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Genetic Polymorphism of IFNA1 and IFNAR1 Genes in Covid-19 Iraqi Patients

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Abstract

Coronavirus disease 2019 (Covid-19) is a deadly acute respiratory illness brought on by an infection with coronavirus 2. (SARS-CoV-2). The severity of the syndrome was variant among the individuals ranging between severe and mild. Interferons type 1 are one of the cytokines inactive the viral replication. Genetic variation in rs2850015 for IFNA1 and rs202055606 for IFNAR1 were investigated by high-resolution melting analysis. Results showed that TT genotype and T allele of rs202055606 polymorphism represented a significant positive odds ratio as risk factor for severity while C allele act as protective factor. The rs2850015 polymorphism revealed conversion T to G, the GG genotype and G allele represented a significant positive odds ratio as risk factor for severity while the T allele represent protective factor from severity. It was be concluded that the GG genotype and G allele for rs2850015 and TT genotype and T allele may be associated with severity of Covid-19.

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1. Introduction

Coronavirus disease 2019 (Covid-19) is a deadly acute respiratory illness brought on by an infection with coronavirus 2. (SARS-CoV-2) [1,2]. Patients with Covid-19 infection experience pneumonia, a dry cough, dyspnea, and a high temperature [3]. Clinical manifestations of Covid-19 vary, ranging from mild sickness to severe disease with cytokine storm. The death rates vary from 0.5 to 13 percent over the world. This variance is probably caused by both host and pathogen influences. Host factors may include variations in the immune response genes' genetic makeup [4].

A cascade of cytokines is released once tissue macrophages recognize viral infections. These cytokines stop virus replication through type I interferons (IFN-a and IFN-b) and assist in triggering the natural killer (NK) cells, the second line of innate immune defense, with their array of activating and inhibitory receptors [4].

Common molecular mechanisms for several viral infections are shared by genetic resistance to severe viral illnesses. The pro-inflammatory response to the introduction of an infectious agent is greatly aided by interferon expression pathways in host cells, and the presence of certain variations in interferon gene loci decreases natural immunity and increases susceptibility to severe viral illness [5]. A vast family of homologous pro-inflammatory, antiviral, immune-regulatory cytokines, the type I interferons, which include the IFNAs, IFNB1, and IFNW1, are encoded by a collection of single exon genes in a 400 kb area of human chromosome 9p21.3 [6].

The susceptibility to and outcome of chronic hepatitis B virus (HBV) infection have been linked to genetic variations within the promoter of interferon- receptor type I (IFNAR1) The main IFNAR-expression transcript's levels are not influenced by the IFNAR-1 promoter polymorphisms [8]. Based on a comparison of Covid-19 and other viral infections, we may conclude that the interferon pathway gene variants may act as markers of the potency of the immune response to various viruses [5].

IFNs-I are the initial line of the immune system's protection from viral infections, along with type III IFNs. When pattern recognition receptors (PRRs) detect viral products, the signal converges on activating the mitochondrial antiviral signaling protein (MAVS) in the case of RNA viruses, such as retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5(MDA5), which are the primary cytosolic receptors for RNA helicases, and this in turn activates the TANK-binding kinase,, which causes IFN-regulatory factors 3 and 7 (IRF3, IRF7) to be phosphorylated and activated. IRFs then go to the nucleus and trigger the synthesis of IFNs-I (IFNα, IFNβ, IFNε, IFNτ, IFNκ, IFNω, IFNδ and IFNζ) [7]. Immune responses play a part and may be linked to the early removal of viruses. As a result, certain therapeutic approaches, such as the early administration of type I interferon therapy, may promote the clearance of SARS-CoV-2 [9]. The timing and kinetics of systemic and local IFN reactions during Covid-19, as well as their respective roles in the pathogenesis and severity of the disease, are still being fully elucidated. In

addition, the dysregulated IFN responses are indicative of the successful immunomodulatory strategies used by beta coronaviruses [10]. Chronic HBV infection is related with the polymorphic IFNA1 (–2) gene variation, and in these individuals, the pathogenesis of chronic infection may be influenced by the differential expression of the IFNAR1 and IFNA1 genes [11].

The findings show that various cytokine and cytokine receptor variations contribute differently to illness in populations living in different malaria endemic locations [12]. According to research on multiple sclerosis, the promoter region of IFNAR1's genetic variability may have an impact on how the body reacts to IFN-β [13]. The IFN-pathway may have a role in the susceptibility to or development of HIV-1 infection [14]. A cascade of cytokines is released once tissue macrophages recognize viral infections. Through type I interferons (IFN-a and IFN-b), these cytokines stop virus replication while also assisting the natural killer (NK) cells, the second innate immune system's defense, the natural killer (NK) cells, with their array of activating and inhibitory receptors [15,16]. Insights into the disease pathophysiology, individual vulnerability to SARS-CoV-2 infection, severity, comorbidities, and mortality prognosis can be gained by understanding the host gene diversity and its relationships with Covid-19. Additionally, this data may aid in the selection of suitable intervention targets [17].

Aims: Investigation for the role of genotype and allelic frequencies of *IFNA1* (–2C → T) and *IFNAR1* (–97T → G) gene polymorphisms and their relation with severity level of Covid-19 patients was the key aim for the current study.

2. Material and Methods

Subject:

Thirty adults' volunteers who had variable Covid-19 infection severity (mild and severe) were included in this study. The severity score was determined previously by estimating IgM and IgG levels, IL6 level, D-Dimer, HbA1C level, LDH, CRP and CBC. Blood samples were collected in EDTA tube from each participant. Then, 200 µl were utilized to extract the DNA from each sample.

DNA extraction and genotype:

Genomic DNA was extracted from white blood cells for Covid-19 patients using Easy Pure Blood Genomic DNA Kit (Trans Gen Biotech, Catalog Number: EE121-01, Beijing, China) according to the manufacturer's protocol. The genomic DNA was obtained then used for genotyping by Real-time quantitative PCR (qPCR) and the high-resolution melting (HRM) method which was carried out on Rotor-Gene (Qiagen) to identify *IFNA1* (rs202055606 C → T, also called rs1065752) and *IFNAR1* (rs2850015 T → G), polymorphisms. HRM-PCR contained 10 µl of 2X TransStart ©Tip Green qPCR Super mix, 1µl of each primer. For rs2850015 (F; 5' CGGTGAGAGCTAAGAGGGG 3' and R5' CATCGCCCGTCCTAAGTC 3'). For rs202055606 (F; 5'ATCTCAGCAAGCCCAGAAGT 3' and R; 5'ACCAGGACCATCAGTAAAGCA 3').Then 3 µl of isolated genomic DNA; 5 µl nuclease free water the following reaction conditions were ;employed: denaturalization at 94 °C for 5sec, followed by 40 cycles at 95 °C for 15 s, annealing at 58 °C for 15 s and a final extension step at 72 °C for 20 s. Raw data obtained were analyzed using Rotor-Gene Q software (version 2.3; Qiagen). The melting conditions were 55-95 °C of gradual warming, with step increases of 0.1 °C every 2 s for dissociation curve formation and a 90-second resting period for premelt conditions. Using the saturating dyes. The primers (Table 1).

Table 1. Primers sequences.

Gene/Rs	Primers	Tm	Size
IFNAR1 rs2850015	Forward	58.88	201
	Revers	59.93	
IFNA1 rs202055606	Forward	59.01	101
	Revers	59.01	

Statistical analysis:

Fisher's exact test P values and the ODDs ratio, which was evaluated using a unique X^2 formula, were both statistically significant, and their statistical significance was estimated using the WINPEPI computer software (version 11.63) [18]. Using WpCalc's chi squared test, the Hardy-Weinberg equilibrium was examined [19].

3. Results

Thirty subjects were divided into two groups severs and mild according to results of severity scoring. According NCBI blast the rs 2850015 polymorphism most appear as T→C, however the current study revealed the polymorphism conversion T → G, three genotypes TT, TG, GG were observed as shown in Figure 1-A. The genotype distribution and anticipated allele frequency between the two groups of severe and mild Covid-19 patients showed extremely significant variations.

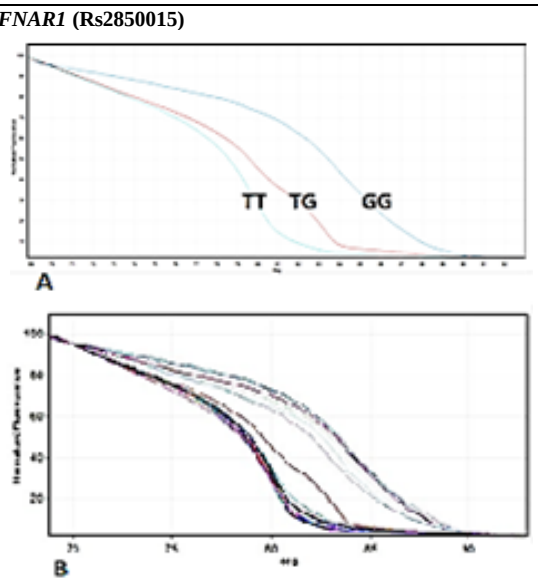

Figure 1. A: rs2850015 genotype in Iraqi samples by HRM method. B: The distribution of genotypes of rs2850015 in severe and mild Covid-19 patients.

According to Hardy Weinberg equilibrium shown in Table 2, the observed and expected frequencies of severe genotypes showed significant differences they departure the H.W.E. While the observed and expected frequencies of mild genotypes showed agreement with H.W.E.

Results in Table 3 showed significant variations between the two groups of severe and mild Covid-19 were discovered in terms of genotype distribution and allele frequency. A significant negative association between homozygous TT genotype with severity and odd ratio was (O.R 0.04) while TG and GG genotypes show positive association with the severity (odds ratio1.33 and infinity respectively). Moreover, the odd ratio of T allele was protective (0.02) and G allele (62.33) demonstrating a positive correlation with severity. It might be regarded as an etiological component that may let severity of disease increase. and could be considered as common genotype in Iraqi population and recorded higher frequency in severe and mild subjects as shown in Table 3.

Table 2. By using Hardy Weinberg Equilibrium, rs2850015 expected genotypes frequencies of severe showed departure from H.W.E.; while the mild group revealed agreement with H.W.E.

Groups / Genotypes		TT	TG	GG	X^2
Severe genotype	Observed no.	4	1	8	8.9571*
	Expected no.	1.5577	5.8846	5.5577	
Mild genotype	Observed no.	16	1	0	0.0156 N.S.
	Expected no.	16.0147	0.9706	0.0147	
Total observed		20	2	8	

(*) The observed and expected frequencies that showed significant differences were ($X^2 \geq 3.84$) for severe and mild groups. (N.S.) Observed and expected frequencies for mild group showed no significant differences.

Table 3. Distribution of IFNAR1 (rs2850015) polymorphism genotypes in Covid-19 patients.

rs2850015 genotypes	Severe No.(%)	Mild No.(%)	Odds ratio	CI95%	Fishr's exact probability *	Attributable fraction	prevented fraction
TT	4 (30.77%)	16 (94.12%)	0.03	0.00 to 0.27	0.000 **		97.2%
TG	1 (7.69%)	1 (5.88%)	1.33	0.03 to 55.07	0.746	25.0%	
GG	8 (61.54%)	0 (0.00%)	infinity	5.09 to infinity	0.000 **	100.0%	
Total 30	13	17					
	Alleles distribution						
T No. (%)	9/26 (34.62%)	33/34 (97.06%)	0.02	0.00 to 0.12	0.000 **		98.4%
G No. (%)	17/26 (65.38%)	1/26 (2.94%)	62.33	8.68 to 1347.83	0.000 **	98.4%	

Significant differences P ≤ 0.05*, while P value > 0.05 considered non-significant.

The second SNP was investigated in current study was rs202055606 polymorphism of *IFNA1* gene; which reveled three genotypes CC, CT and TT at locus 9:21440506 on chromosome 9 as seen in Figure 2A.

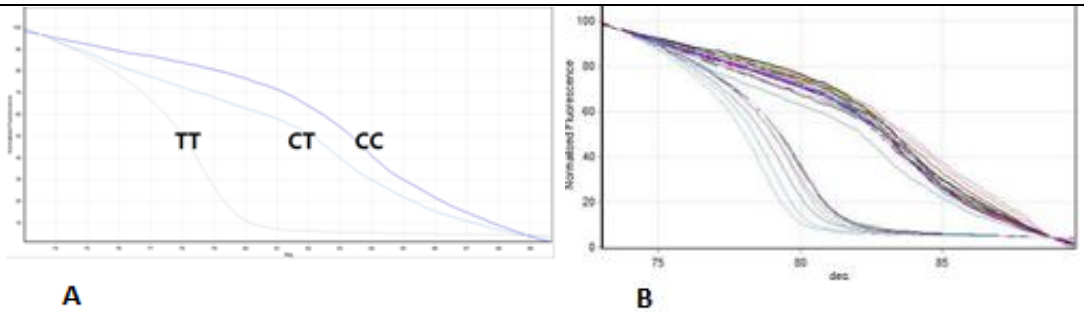

Figure 2. A: rs202055606 genotype in Iraqi samples by HRM method. B: The distribution of genotypes of rs202055606 5 in severe and mild Covid-19 patients.

The observed genotype frequencies exceeded those expected by the Hardy-Weinberg Equilibrium by a significant amount, it was 13 in severe with no significant differences in mild group 3.6022, respectively (Table 4); this departure from HWE may be attributed to the severity of disease. The significant differences that revealed among genotype distribution and alleles frequency between the two groups severe and mild Covid-19 respectively. The genotype CT was not excised in server group at all with present in mild group. In one hand, significant genotype homozygous CC show negative association with the severity (odds ratio 0.28) while TT genotype shows positive association with the severity (odds ratio12.0 respectively). Furthermore, the T allele's odds ratio was 0.16; hence, a protective allele may be thought of as having a negative association with severity. While the odds ratio for the G allele was 6.17, demonstrating a positive association between the allele and severity, It might be regarded as an etiological component that may let severity of disease increase. While the total observed results (sever and mild) shown in Table 5 indicate that CC homozygote genotype is the common genotype at this locus. The frequency of CC was (5+12) mild and severe infection.

Table 4. By using Hardy Weinberg Equilibrium, rs 202055606 genotypes expected frequencies of sever showed departure from H.W.E while the mild group revealed agreement with H.W.E.

Groups		CC	CT	TT	X^2
Severe genotype	Observed no.	5	0	8	13*
	Expected no.	1.9231	6.1538	4.9231	
Mild genotype	Observed no.	12	3	2	3.6022 N.S.
	Expected no.	10.7206	5.5588	0.7206	
Total observed		17	3	10	

(*) The observed and expected frequencies that showed significant differences were ($X^2 \geq 3.84$) for severe group. (N.S.) Observed and expected frequencies for mild group showed no significant differences.

Table 5. Distribution of IFNA1rs202055606 (rs1065752) polymorphism genotypes in Covid-19 patients.

Rs202055606 genotypes	Severe No. (%)	Mild No. (%)	Odds ratio	CI95%	Fisher's exact probability *	Attributable fraction Etiological fraction	prevented fraction
CC	5 (38.46%)	12 (69.23%)	0.28	0.05 to 1.49	0.142		72.2%
CT	0 (0.0%)	3 (17.65%)	0.00	0.00 to 2.16	0.154		17.6%
TT	8 (61.54%)	2 (11.76%)	12.00	1.84 to 96.20	0.004**	91.7%	
Total 30	13	18					
	Alleles distribution						
Cn (%)	10/26 (38.46%)	27/34 (79.41%)	0.16	0.05 to 0.52	0.002 **		83.8%
Tn (%)	16/26 (61.54%)	7/34 (20.59%)	6.17	1.92 to 19.98	0.002 **	83.8%	

Significant differences $P \leq 0.05$* while P value > 0.05 considered non-significant.

4. Discussion

The current study investigated the association of the polymorphism rs 2850015 with severity for Covid-19 infection showed the GG genotype and G allele as factors may have a role to encourage severity of Covid-19. According NCBI blast the rs 2850015 polymorphism most

appear as T > C, in the current study the polymorphism reveals conversion T > G may be ethnic differences from other population, which represents sample of Iraqi population. The genomic region rs 2850015 polymorphism of *IFNAR1* gene on chromosome 21 represents the 5' regulatory region of the interferon alpha and beta receptor subunit 1, which encodes an antiviral factor that may had a role in interferon immunity in Covid-19 [20]; This region functions as a promoter that drives IFNAR1 expression. It includes a binding site for the high mobility group box 1 (HMGB1) protein, which together with poly(ADP-ribose) polymerase 1 (PARP-1), functions as a cofactor for IFNAR1 transcription, and where suppression of PARP-1 by hepatitis B virus infection can lower IFNAR1 expression [20]. According to NCBI clinical variation has not been recorded for rs2850015, but some studies mention to important of this region in somehow. Patients with multiple sclerosis who have IFN-responsive genetic variability in IFNAR1's promoter region [13]. Multiple polymorphisms have been found within this promoter, the promoter of *IFNAR1*gene located in LOC119230225gene, including a SNP (rs2850015) that affects promoter activity in HepG2 liver carcinoma cells [4,21].

In the other hand rs202055606 which had merged into rs 1065752 and located at Chromosome 9 at the locus 21440506, this region represents 5' UTR variant of *IFNA1* gene. According to NCBI rs1065752 has not been reported to clinical variation. Unlike the role of C allele in Hepatitis virus infection as a risk factor of chronicity and T allele which associated with clearness of HBV, the current study presented information that the TT genotype and T allele which had high significant positively associated with severity it may serve as a risk factor for the severity of Covid-19 disease. No reports have been published previously on these polymorphisms in promoter region of gene that associated with Covid-19.

Finally, variable disease severity might be caused by varied immune response kinetics brought on by polymorphisms in the various innate and adaptive immune response components. It is essential to comprehend how this genetic difference may change how the body reacts to SARS-CoV-2 infection in order to create effective treatment methods [4].

5. Conclusions

The mutant genotype GG and G allele of rs2850015, mutant TT genotype and T allele in rs202055606 could be used as molecular biomarker of susceptibility to severity of Covid-19 infection.

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