Human FcγR2A Genotype is Correlated with Sever Clinical Outcomes of SARS-COV2 infection

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ABSTRACT

Human Fc gamma-receptor II alpha (FcγRIIA) Polymorphism has been linked with predisposition to susceptibility and/or severity degrees of several infectious diseases. To find if there is any link between these genetic polymorphisms and the intensity of the severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) disease. FcγRIIA polymorphism has been evaluated in the DNA extracts of 100 SARS-Cov-2 patients from Iraq. The participants included 74 mildly and moderately infected patients and 26 severely infected patients. A significant correlation was detected among FcγRIIA-131 polymorphism and severity of COVID-19, with higher frequency of FcγRIIA G/G 131 homozygote in severely infected individuals compared to that in the mildly/moderately infected patients in codominant and recessive mode; ($p = 0.004$ and $p = 0.003$), respectively. While AG and AA genotype could act as a protective factor. The outcomes demonstrate that FcγRIIA polymorphic genotype may affect the severity of the SARS-Cov-2

Keywords: COVID-19, FcγRIIA polymorphism, Genetic variation, Iraqi population

Introduction

Current novel coronavirus disease 2019 (COVID-19) caused by SARS-COV2, is the midst of worldwide panic and global health concern since December 2019, it has been expanded dramatically throughout the world and became an important global crisis and battle against this deadly virus (Guan et al., 2020). COVID-19 disease was first documented in Wuhan, Hubei city in China at 17th of December 2019, then it had been extended more rapidly throughout the world in a dramatic manner causing a devastinting impact in most of the countries. Due to its contagiousness and easy of transmission, which result in recording a high number of persons whom severely sick and need a hospitalization in addition to elevated risk of death (Garcia, 2020), the World Health Organization (WHO) characterized the infectious illness as a major health problem on 30 of January 2020 and a novel pandemic on the 11th of March 2020 (WHO, 2020). Pathological

consequences of SARS-COV2 infection exhibit a wide variant of clinical symptoms that range from asymptomatic disease, mild or common cold, to moderate and severe outcomes **(Mo P *et al*., 2020).** The most common sign and symptoms of COVID-19 are: fever, headache, dry cough, shortness of breath, myalgia and fatigue **(Chen N *et al*., 2020).** Some patients presented with gastrointestinal outcomes such as: vomiting and diarrhea**.** Major complications that can be observed in severe cases are: SARD, acute lung injury (ALI), kidney injury, arrhythmia and cardiomyopathy, multi-organ failure especially in elderly people, thrombosis and pulmonary embolism which always associated with higher level of fibrinogen and D-dimer **(Azer S, 2020)**. Host genetic differences are well recognized to contribute to variable immune response among persons infected with SARS-Cov-2. It is clear that host genetic factors could affect the susceptibility and severity of a range of infectious diseases and their clinical manifestation via selection of suitable candidate gene depending on pre-knowledge about pathogenesis and phenotype of the disease (Di Maria *et al*., (2020).). One of the most useful approach in severity and prognosis of COVID-19 is the assessment of innate immune response and humoral immunity (Farag *et al*., 2020). Fragment crystallizable (Fc) receptor is a protein found on the surface of some immune cells like: NK, macrophage, eosinophil, neutrophile, mast cell and platelet. It has a significant role in infectious and inflammatory disease. Fcγ receptor family involves three groups: FcγI, FcγIIa/b and FcγIIIa/b. Its name derived from binding to Fc domain of immunoglobulin IgG which already bound to pathogen or infected cells, thus activating cytotoxic or phagocytic cells to destroy microbes or induce Ab dependent cell mediated cytotoxicity (ADCC) (Patel *et al.,* 2019). Human FcγRIIA (CD32, as another name) is an essential member within Fc receptor family, has a critical role in immune reaction regulation, auto-immune disease, and regulation of local inflammation also forms an important link between the humoral immunity and cells of adaptive immunity. FcγRIIA receptor-encoding genes known to have a functional SNPs at 131 amino acid residues with a *G to A* point mutation result in either arginine (R) or histidine (H) on Ig-like domain at 131a.a. Genotypes of FcγR have been reported to affect the binding to/and affinity for IgG isotype of FcγR. The polymorphism variation will affect the function of these receptor through interfering with their trafficking and localization of cells that suggested to be involving the pathogenesis processes of infectious, autoimmune and inflammatory disease (Junker *et al.,* 2020). Expression of *FcγRIIA* gene is increased by environmental cytokine like: IFN-γ, IL-3 and -6, C5a, and dexamethasone and reduced by IL-4, TNF-α, and β (Anania *et al*., 2019). The work, here, was conducted to find if *FcγR2A* polymorphic variants have any correlation with the COVID-19 pathogenesis and severity.

Materials And Method

Study Design and Data Collection

One hundred subjects with confirmed positive SARS-COV2 based RT-PCR, have been recruited from Al-Diwaniyah teaching hospital, Iraq in period of 1st December, 2020 to the end of January, 2021. Both genders were included. According to infection's severity, the participant individuals have been divided into two subgroups: mild/ moderate group includes 74 patients and severe group contains 26 patients.

Collection of Blood Sample and DNA Extraction

Two ml of peripheral blood sample was drowning aseptically from each infected person and collected in sterile EDTA tubes for DNA extraction and gene polymorphism analysis. DNA extracted from blood via "G-spin™ Mini Kit" (iNtRON, Korea) and done depending on company instructions. The DNA was tested by employing a Nano drop spectrophotometer to measure the concentration of DNA and check the purity, then kept at −20˚C to be used later on.

Polymerase Chain Reaction (PCR)

The PCR was done with set of Primers that have been designed to amplify the 343 bp region of *FcγRIIA* rs1801274, these primers: "F 5′ GGA AAA TCC CAG AAA TTC TCG C 3′ and R 5′ GGA AAA TCC CAG AAA TTC TCG C 3′". Initial denaturation of DNA occurs at 95 °C for 5 min.

for one cycle. Reaction conditions have been set as: denaturation for 30 sec. at 95 °C, annealing at 57.6 °C for 30 sec. and extension at 72 °C for 30 sec. for 35 cycles following by one final extension cycle at 72 °C for 5 min. The PCR products seen in figure 1

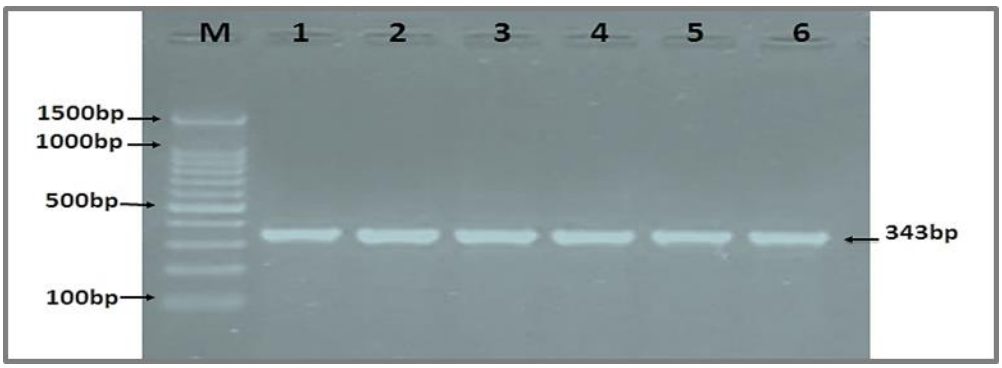

Figure 1: Agarose gel-electrophoresis image of PCR products analysis of *FcγRII* rs1801274 gene polymorphism. M: marker (1500-100bp)

Restriction Fragment Length Polymorphism reaction (RFLP)

Genotyping of *FcγRIIA131A* gene done by using *BstUI* restriction enzymes (Chatzikyriakidou *et al*., 2015). 14µl of PCR product was digested with 1µl-restriction enzyme within master mix tube (New England Biolabs. UK), then placed in Exispin vortex and centrifuged for 2 minutes at 3000 rpm and then incubated at 37°C overnight. Product of RFLP was analysis by 2% agarose gel electrophoresis technique. The products of FcγRIIA131A polymorphism are; 343 bp band of A/A homozygous wild type, GG homozygote mutant type that digested into 322bp and invisible 21bp bands and finally A/G heterozygote products digested into 343bp, 322bp, and non-visible 21bp bands. The products seen in figure 2.

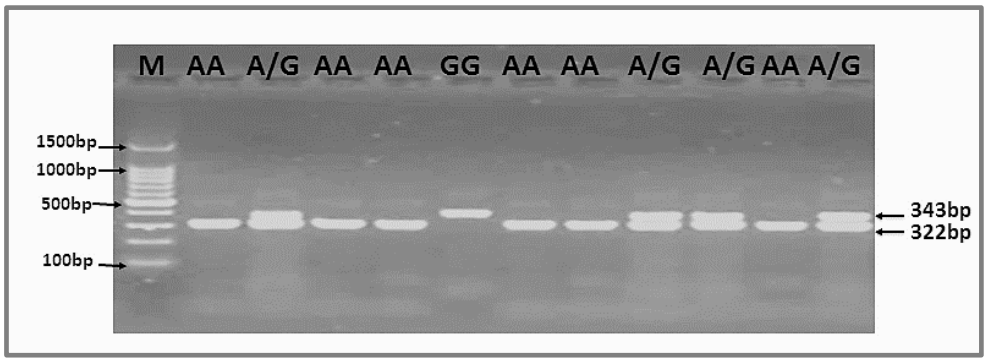

Figure (3-11): Agarose gel-electrophoresis analysis of PCR-RFLP product of *FcγRIIA131*rs1801274 gene polymorphisms. M: marker (1500-100bp)

Statistical analysis of data

Data have been analyzed using SPSS V20. Parametric tests have been used to assess differences in median among 2 groups with Mann-Whitney equation. Spearman's rank linear correlation coefficient has been used to measure the relation between two quantitated variables. Odd ratio (OR) and 95 % of confidence interval (CI) measured by Chi-squared (C) test. *P* value at ≤ 0.05 was used for significance determination. Mean (M) plus/minus standard deviation (SD) was utilized.

Results

Characteristics and demographic parameters of participants

Mean age of all individuals was 42.26 ±13.54 years with wide range of age (10-66 years). Regarding disease severity, no significant difference in mean age was determined between mildly/moderately affected patients and severely affected patients (p = 0.253). However, patients of more than 60 years were more frequently seen in severe disease group than in mild/moderate group (19.2 % versus 6.8 %), respectively (table1). Based on gender, the study involved 75 and 25 males and females with ratio of 3:1, respectively. Linked with severity of infection the females were more frequently seen in the severe category than in mild/moderate group (38.5 % versus 20.3 %), respectively.

Table 1: Frequency distribution of age in patients' subgroups

	Mild to moderate ***n*** **= 74**	**Severe** ***n*** **= 26**	**Total** ***n*** **= 100**	***p***
Age (years)				
Mean ±SD	41.35 ±13.17	44.87 ±14.50	42.26 ±13.54	0.253 I NS
Range	11- 65	20- 64	11- 65	
≤ 20, *n* (%)	2 (2.7 %)	1 (3.8 %)	3 (3.0 %)	0.769 C NS
21-40, *n* (%)	33 (44.6 %)	9 (34.6 %)	42 (42.0 %)	0.375 C NS
41-60, *n* (%)	34 (45.9 %)	11 (42.3 %)	45 (45.0 %)	0.748 C NS
> 60, *n* (%)	5 (6.8 %)	5 (19.2 %)	10 (10.0 %)	0.068 C NS

***n*: number of cases, SD: standard deviation, I: independent samples *t*-test, C: chi-square test, NS: not significant at $p > 0.05$**

Frequency Distribution of FcγR2A rs1801274 SNPs in COVID-19 Iraqi patients

According to Hardy-Weinberg equation, the difference in *FcγR2A* rs1801274genotype distribution between expected and observed counts was substantially significant ($p < 0.001$), table 2

Table 2: The frequency distribution of patients according to Hardy Weinberg equation

FcγR2A-131 **rs1801274**		
Genotype	**Observed count**	**Expected count**
AA	39	29.2
AG	30	49.7
GG	31	21.2
χ^2	15.692	
p	< 0.001 HS	

HS: highly significant at $p \leq 0.01$; NS: not significant at $p > 0.05$

Association of FcγR2A rs1801274 SNPs with COVID-19 severity

Codominance mode has shown a highly significant difference between severe and mild/moderate group (p = 0.004) in such a way that genotype AG is a protective factor (OR = 0.54) and GG is risk factor (OR = 2.26). In dominant mode, the difference was significant (p = 0.019) and genotype AA was a protective factor (OR = 0.20). Recessive mode shows a highly significant difference (p = 0.003), genotype GG was a risk factor (OR = 3.91). Regarding alleles, the difference was clearly significant ($p < 0.001$) and allele G was a risk factor (OR = 3.7), while allele A was protective allele (OR = 0.27), table 3

Table 3: Association of *FcγR2A* rs1801274 gene polymorphism with COVID-19 severity

Mode	*FcγR2A* rs1801274 SNPs	Severe *n* = 26	Mild/moderate *n* = 74	*p*	OR	95% CI	EF	PF
Codominant	AA	4 (15.4 %)	35 (47.3 %)	0.004 CHS	Reference			
	AG	8 (30.8 %)	22 (29.7 %)		0.54	0.21-1.40	---	0.12
	GG	14(53.8 %)	17 (23.0 %)		2.26	0.77-6.63	0.25	---
Dominant	AA	4 (15.4 %)	35 (47.3 %)	0.019 CS	0.20	0.06-0.65	---	0.40
	AG+GG	22(84.6 %)	39 (52.7 %)		Reference			
Recessive	AA+AG	12(46.2 %)	57 (77.0 %)	0.003 CHS	Reference			
	GG	14(53.8 %)	17 (23.0 %)		3.91	1.52-10.04	0.34	---
Allele	A	16(30.8 %)	92 (62.2 %)	< 0.001 CHS	0.27	0.14-0.53	---	0.29
	G	36(69.2 %)	56 (37.8 %)		3.70	1.88 -7.27	0.29	---

n: cases number, **NS**: non-significant if $p > 0$, OR; odd ratio, C; chi square-tests, CI; confidence interval

Discussion

SARS-Cov-2 infection symptoms might be mild, moderate, or severe, and in some cases being critical. For decades, researchers have focused on the role of host genetic variants in human vulnerability and/or disease progression. Unlike other human genes where genetic variation has no clear functional contributions on illness profile, it is well documented that the 1q23 Locus, where the FcR2A gene is located, is associated with various infectious and autoimmune illnesses and the *FcγR2A* shows a clear functional difference between G and A 131 allele type. Antibody dependent cellular cytotoxicity (ADCC) might be regarded as an extra-cellular killer method leads to elimination of microbes (Sananez *et al.,* 2020). IgG exhibits a bi-functional configuration represented by antigen-binding Fab and Fc fragments of antibody. Adhesion of the antibody's Fab and Fc fragments to the pathogen and the FcR on effector cells initiates the ADCC cascade. Consequently, effector cells degranulation results in lysis of pathogen (Lo Nigro *et al*., 2019). The *FcγR2A* genes known to have a functional SNP at 131 a.a causing G to A point mutation called *H131R* or rs1801274 SNP. This mutation results in either arginine or histidine at 131 position in Ig- like domains of antibodies. The receptors encoded by *H-131* (*A 131*) allele show higher affinities for IgG 1, 2 and 3 subtypes than receptors encoded by *R 131* (*G 131)* allele (Bohmwald *et al*., 2019). FcRIIA-H131 receptors' phagocytic ability is boosted by the high binding affinity. Consequently, scientists have examined the correlation between H131R and its possible involvement in protecting against several illnesses. The first indication that *H131* allele could be involved in protection against an infectious disease came from observing differences in allele frequencies between Asian and European populations with susceptibility to Malaria (Amiah *et al*., 2020). The study's findings show for the first time that the intensity of COVID-19 illness might substantially related to the FcR2A-G/G131

genotype (P<0.05). In addition to age and gender, FcγRIIA polymorphic variations were still significant because their frequency differed between the severely and mildly/moderately affected patients (P<0.05). According to our findings, more serious SARS-Cov-2 infection outcomes may be associated with G/G gene, whereas a better outcome for SARS-Cov-2 illness may be associated with A/A genes.

The *FcγR2A*-rs1801274 polymorphisms are well known for its impacts on autoimmune thyroid disease (AITD) and Hashimoto's thyroiditis (HT) (Mestiri *et al*., 2020). Also, it has been reported that RR genotype is association with severity of SARS infection, Yuan et al., (2005) found that RR genotype is highly significant in ICU patient infected with SARS virus compared with control (23% vs 9%, p = 0.03). Alagarasu et al., (2015) also observed a significant higher frequency of R/R genotypes in individuals infected with dengue virus suffering from thrombocytopenia (p < 0.005). On the same line, a study from China done by Qu et al., (2020) suggested that subjects with131R genotype show higher frequency to be attacked by inhibitor development in Hemophilic A (HA) patients. On the contrary, Paul et al., (2019) found that *FcγR2A*-131RR polymorphous was correlated with low rate of chronic lung allograft dysfunction. Also, other study by Holgado et al, (2020), found that the presence of *FcγRIIA*-131/HH genotype has been observed in higher frequency among children infected with severe RSV- infected children comparing with moderately infected children. Wang et al., (2020) also found that *FcγR2A*-131A/A "rs1801274" was strongly associated with Kawasaki disease (KD) occurrence, and A allele might raise the incidence rate of disease than G allele. Zakaria et al., (2021), they also suggested that the *FcγR2A*-131 H and R allele frequency was 51.3 % and 48.7%, respectively in children suffering from primary immune thrombocytopenia (ITP) versus 75% and 25%, in controls (p = 0.002). Regarding that the immune complex can has a potential pathogenic effect and contribute to tissue damage, the higher affinity of H/H gene, instead of resolution role, may actively causes tissue damage via inducing an exacerbated immune response and enhancement the infection process.

Conclusions

The major finding of the study is a positive correlation between *FcγRIIA*-R/R gene and severe infection, while A/A might have a protective role against severe prognosis. The interest of this study is to provide a vigilant surveillance and good successful management for individuals infected with SARS-Cov-2.

Recommendations

In view of the limitations of the study such as: ethnic variation, general conditions of the study and relatively small sample size and number of mild cases (only 4 mild cases therefore joined with moderate patients), further studies are recommended with larger group of patients Including various ethnicities to validate these current findings.

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Ethics Consideration

Protocol for this study was ethically approved by the Committee of Iraq Ministry of Health and Environment. Oral approval has been taken from all participants before taking the blood samples.

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