Molecular Analysis of CD147 Gene among Sars-Cov2 Patients and Vaccinated People in Babylon Province.

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Abstract

SARS-COV2, a beta coronavirus, the virus that caused the epidemic of coronavirus illness in 2019 (Covid-19) has been discovered. in late 2019. Corona viruses various proteins have been identified. Discovered as interactors between SARS and CoV-2, and Their capacity to engage with host membrane receptors is due to spike proteins. SARS-COV attaches to cyclo philin A and identifies the CD147 receptor present on the surface of host cells (Cy PA), a ligand for CD147, via its nucleo capsid protein. The study aim to investigate the distribution and detection of SNPs CD147 gene mutations in Covid-19 patients as a receptor for virus entry and a modulator of virus entry through endocytosis. Twelve samples were shown to amplify CD147 gene. The study's findings showed that there is a considerable variance in the rates of mutations for a gene CD147, and the results of the sequencing analysis of the studied segment revealed 229 bp of the gene CD147 the presence of substitution and insertion mutations is distributed unevenly according to their positions P151, p (220-221) in the samples that were studied.

Key words: SARS-CoV-2, CD147 Gene, Iraqi Human, PCR, Nucleotide Sequences

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1. Introduction

On December 31, 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in Wuhan, Hubei Province, China. SARS-CoV-2 is a new coronavirus that has emerged in 2019 (COVID-19) Dehghanbanadaki et al. [1] [2]. owing to the strong Angiotensin-converting enzyme {ACE2} affinity of SARS-CoV2 spike proteins and viral load, SARS-CoV2 has become widespread considerably SARS-like speed [3].

This corona-virus family's capacity to connect with receptors produced on host cell membranes has been linked to the spike protein [4],various SARS-CoV2 Spike protein interactors have been discovered, including Angiotensin-converting enzyme2 {ACE-2} [5], Neuro pilin -1 {NRP1} [6, 7], and Basigin2/EMMPRIN/CD147 (CD147. In many types of human cells, The ACE-2 receptor (angiotensin-converting enzyme 2) has been discovered found to give an example entrance site for SARS-COV 2 [4, 8]. T cells, on the other hand, have been found to be ACE-2 receptor=negative on a consistent basis [9], implying a different path of viral invasion. Recently, CD147 was offered as a candidate possible pathway in the case of SARS-CoV2 infection of Wang [10] discovered Vero E6 host cells. CD147 (also known as Basigin or EMMPRIN) is a transmembrane glycoprotein that was first discovered in 1992 as a T cell activation antigen [11]. Moreover studies revealed that it is expressed on activated T lymphocytes but not on T cells at rest, and that its expression rises. fast when T cells are activated [12]. Inflammatory response, wound healing, and tissue homeostasis are examples of physiological and pathological phenomena.CD147 stimulates the formation of matrix metalloproteinase (MMP) [13-16].CD-147 is a 58-kDa immunoglobulin superfamily cell surface glycoprotein that was first discovered on the surface of tumor cells, where it can promote MMP synthesis in nearby fibroblasts via homotypic CD147–CD147 interactions. (Yan L. et al., 2005; Suzuki S. et al., 2004) CD147 has been found as a simple marker of inflammation, despite the fact that the pathogenic mechanisms of CD147 are unknown [17, 18].

As a result of the major significance of SARS-CoV-2 infection and the role of CD-147 antiviral impact Peptide-9, a CD147 antagonist, multiple studies have been conducted [19]. According to CD147 is engaged in the indirect contact between cyclo-philin A and viral spike protein, according to surface plasmon resonance, co-immunoprecipitation, and enzyme-linked immunosorbent assay (ELISA) studies, and it also binds directly to the viral S protein with a considerable high affinity [20]. Wang, K et al. published the initial study of the interaction between SARS-CoV-2 Spike protein and the host cell receptor CD147 at the end of 2020, showing that modulating receptor levels influenced the virus's capacity to infect the cells being infected. They also discovered CD147 receptor exists is important in immune cells that do not express ACE2, and proposed this pathway as a potential entry point for SARS-CoV-2 infection [21].

2. Materials and Methods

Blood sampling

Blood samples were collected from a selection made at random from Iraqi human coronavirus patients and vaccinated subjects selected from the hospital in Babylon province. About 3 ml of blood samples by jugular vein puncture using disposable needle for the period from the first of October 2021 to February 2022.

DNA Extraction

DNA was extraction from peripheral blood using genomic DNA extraction kit (Favor gen - Taiwan). A nanodrop (BioDropLITE, Biodrop, UK) was used to evaluate the concentration and purity of DNA after it was extracted.

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The DNA degradation probability was then determined using a conventional percent (w./v.) electrophores-s on an agarose-gel with ethidium bromide pre-staining (1 µg/mL) in TBE buffer.

PCR Amplification:

The CD147 gene was PCR-amplification using one pair of primers that are specific. The Primers were desired according to(GenBank acc.No.NG_007468.1). The lyophilized primers were purchased from Macrogene (Korea). The sequence of Forward: 5'GCTCTGCACCCCTGTAAGTT3' and Reverse: 5'CAGCACCAGAATGACAAGG3'.PCR the reaction was carried out employing Master Mix(Pro-mega). The gradient PCR thermocycler was used to start the program was began by initial d-naturation at 92°C for 2 minute was 30 cycle of denaturation at 92°C for 30 second, annealing at 55°C for 30 seconds, and elongation at 72°C for 30 second, followed by 3 minute of final extension at 72°C in a gradient PCR thermocycler. Resolving the PCR amplified products on 1.5 percent agarose in conjunction with a 100 bp DNA ladder validated the PCR amplified products. Gel electrophoresis was carried out at a constant voltage in 1X TBE buffer of 100 V for 45 minutes. It was ensured that all PCR determined bands were distinct and only contained one band (229bp fragment each).

DNA sequencing

Twelve samples were sent to Microgene Company for the purpose of genetic analysis to compare it with the standard sources in NCBI.

3. Statistics for Analyses

To detect the effect of difference factors in study percentage, [22] was used. The Chi-square test was used to conduct this research compare percentages (0.05 and 0.01 likelihood).

4. Results and Discussion

Genotypes were defined based on the visualization of various band patterns. Using the primers used for amplification, the PCR-derived products belonging to Different designs were sequenced in a unique way.

Using Bio Edit Sequence Alignment Editor Software Version 7.1, the sequencing results of various PCR products were edited, aligned, and assessed as long as they matched the appropriate sequences in the reference database (DNA STAR, Madison, WI, USA). Each sequenced sample's detected differences were numbered in PCR amplicons and their matching positions within the referring genome. The PCR amplified sequence of CD147 for sequence homology searches in public databases, the query gene was run through NCBI's nucleotide blast (http://blast.ncbi.nlm.nih.gov./blast/Blast.cgi).In this study, CD147 gene was studied for the Homo sapiens. PCR was utilized to boost a 229-bp a section of CD147 gene 's of the part from exons 7 and 8, as well as the intervening intron (Fig. 1).

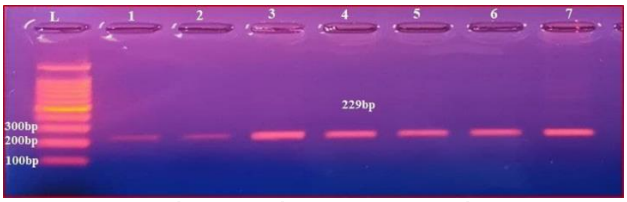


Fig. 1: Amplification of CD147 (229 bp fragment) by PCR in 1.5% agarose gel, 100 bp DNA ladder.

The current study comprised twelve samples from this locus that had previously been demonstrated to amplify CD147 gene sequences in human chromosome 19. After running these PCR amplicons through NCBI blastn (https://blast.ncbi.nlm.nih.gov/Blast.cgi),, the sequencing reactions revealed the exact identity. The NCBI BLASTn engine found 99 percent sequence similarity between the sequenced samples and the target sequences that will be used as a reference for the alleged 229 bp amplicons. The estimated locations as well as features a obtained By comparing the observed DNA sequences of these local samples with the returned DNA sequences,PCR fragments were found (GenBank acc.no. NG-007468.1) (Fig. 2).

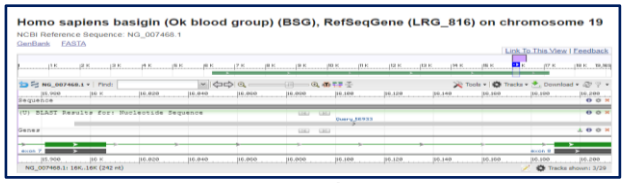
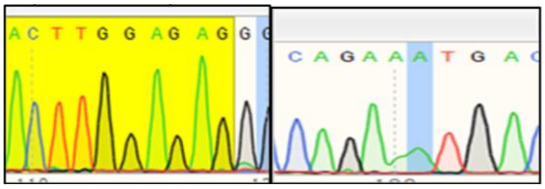


Fig. 2: The precise location of the 229 bp amplicon that partially encompassed a region of the genome that was a part of the cost CD147 gene on chromosome 19 (GenBank accession number NG 007468.1).

The detected substitution and insertion SNPs' sequencing chromatograms, additionally their extensive annotation's, were reported, as well as the observed chromatogram detail's SNPs were displayed based on their positions in the PCR amplicons. (Fig. 3).



P 151: A>G P 220-221: A ins.

Figure 3: shows the substitution pattern mutations found in the targeted target's DNA chromatogram 229 bp amplicons in the human CD147 gene. The sites of the detected substitution and insertion mutations in the PCR products were highlighted. S1 – S12 refer to the studied no. 1 to no. 12 samples. The symbol ">" refers to the mutation event, while the phrase "ins" refers to insertion mutation.

To summarize all of the findings from the 229 bp fragments that were sequenced, in the NCBI reference sequences, the specific sites of the detected changes were given. (Table 2).

Table 2: The observed a pattern SNPs in the 229 bp amplicons of Homo sapiens (vaccinated and infected population) in comparison with their corresponding Referring sequences from the NCBI (GenBank accession number NG 007468.1). The letter "S" stands for sample number.

Population/no.	Sample no.	Native	Allele	Position in the PCR
Vaccinated/4	2	A	G	151
	1	A ins.	-	220-221
Infected/8	5	A	G	151
	2	A ins.	-	220-221

The observed variants had demonstrated two in the examined samples, distinct distributions in terms of the targeted 229 bp amplicons.

The study's findings showed that there is a considerable variance in the rates of mutations for a gene CD147, the percentage of mutations in the infected and the vaccinated was 87.50%,75.00% respectively (Table 3).

Table 3: Distribution of sample study according Mutation results in Vaccinated and Infected.

Group	Total No	Mutation No. (%)	No Mutation No. (%)	χ2 (P-value)
Vaccinated	4	3(75.00%)	1 (25.00%)	1.0000 (0.3173)
Infected	8	7 (87.50%)	1 (12.50%)	4.5000 * (0.0339)
Total	12	10(83.33%)	2 (16.67%)	5.3333 * (0.0209)
* (P≤0.05)				

The results of the sequencing analysis of the studied segment revealed 229 bp of the gene CD147 the presence of substitution and insertion mutations is distributed unevenly according to their positions P151, p (220-221) in the samples that were studied (Table 4).

Table 4: Distribution of sample study according to type of Mutation in Vaccinated and Infected

Group	Type of Mutation	No	%	χ2 (P-value)	Position in the PCR
Vaccinated (No= 4)	AG	2	66.67	0.3333 NS (0.5637)	151
	A ins.	1	33.33		220-221
	Total	3	100%	--	--
Infected (No= 8)	AG	5	71.43	1.2857 NS (0.2568)	151
	A ins.	2	28.57		220-221
	Total	7	100%	--	--
NS: Non-Significant.					

This genetic difference is present in the non-coding DNA (the seventh intron) at site 151 and the change was from UCA Serine (AGT) to Proline CCA (GGT), and the genetic difference in the eighth axon at the position 220-221 changes the amino acid sequence in this region of the peptide, since this SNP is an insertion mutation and the change was from Tyrosine UAC (ATG) to Leucine UUA (AAT) and thus led to a change in the amino acid sequence causing the amino acid sequence of CD147 protein a replacement effect.

Showed to the sequence identity of the NCBI homolog of the CD147 gene for local samples versus Ref seq. CD147 gene (accession number NG_007468.1). Below (Table 5), for vaccinated people, the nucleotide polymorphisms and amino acid polymorphisms to the (GAG > GGG) Glutamic acid > Glycine, and the (AAA) Lysine for the samples (S.9, S.10, S.11, S.12) and the polymorphism type was the percentage of differences (1.11, 0.56) and the percentage of identity (99.44, 98.89). And for the injured the nucleotide polymorphism and Amino acid polymorphism to the (GAG > GGG) Glutamic acid > Glycine, and the (AAA) Lysine to the samples (S.1, S.2, S.3, S.4,S.5,S.6,S.7,S.8) and the Type of polymorphism Percentage of variations (0.0,1.11, 0.56) and Percentage of identity(99.44,98.89,100). As a result, the amino acid sequence of CD147 protein was replaced.

Table 5: NCBI homology sequence identity of CD147 gene of local samples against Ref Seq. CD147 gene (accession No. NG_007468.1).

Sample No.	NCBI homology sequence identity				
	*Nucleotide polymorphism	**Amino acid polymorphism	Type of polymorphism	Percentage of variations	Percentage of identity
Sample 1	GAG>GGG	E>G	Missense	0.56	99.44
Sample 2	GAG>GGG AAA	E>G K	Missense Insertion	1.11	98.89
Sample 3	GAG>GGG	E>G	Missense	0.56	99.44
Sample 4	Non	Non	Non	0.0	100
Sample 5	GAG>GGG	E>G	Missense	0.56	99.44
Sample 6	AAA	K	Insertion	0.56	99.44
Sample 7	Non	Non	Non	0.0	100
Sample 8	Non	Non	Non	0.0	100
Sample 9	GAG>GGG AAA	E>G K	Missense Insertion	1.11	98.89
Sample 10	GAG>GGG	E>G	Missense	0.56	99.44
Sample 11	GAG>GGG AAA	E>G K	Missense Insertion	1.11	98.89
Sample 12	GAG>GGG AAA	E>G K	Missense Insertion	1.11	98.89
*(G= Guanine, C= Cytosine, T= Thymine, A= Adenine) **(G= Glycine, E= Glutamic acid, K= Lysine)					

5. References

1. Dehghanbanadaki H, Seif F, Vahidi Y, Razi F,

Hashemi E, Khoshmirsafa M, Aazami H. Bibliometric analysis of global scientific research on Coronavirus (COVID-19). Medical journal of the Islamic Republic of Iran. 2020;34:51. https://doi.org/10.34171%2Fmjiri.34.51

2. Nezhad MS, Seif F, Darazam IA, Samei A, Kamali M, Aazami H, Mohsenzadegan M, Mollaei-Kandelousi Y, Babaheidarian P, Khoshmirsafa M. An overview of the prominence of current diagnostic methods for diagnosis of COVID-19. AIMS Allergy and Immunology. 2020;4(3):60-74. https://doi.org/10.3934/Allergy.2020006

3. Seif F, Aazami H, Khoshmirsafa M, Kamali M, Mohsenzadegan M, Pornour M, Mansouri D. JAK inhibition as a new treatment strategy for patients with COVID-19. International archives of allergy and immunology. 2020;181(6):467-75. https://doi.org/10.1159/000508247

4. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, Wang Q, Xu Y, Li M, Li X. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharmaceutica Sinica B. 2020;10(5):766-88. https://doi.org/10.1016/j.apsb.2020.02.008

5. Yan C, Liang L-J, Zheng K-Y, Zhu X-Q. Impact of environmental factors on the emergence, transmission and distribution of Toxoplasma gondii. Parasites & vectors. 2016;9(1):1-7. https://doi.org/10.1186/s13071-016-1432-6

6. Cantuti-Castelvetri L, Ojha R, Pedro LD, Djannatian M, Franz J, Kuivanen S, van der Meer F, Kallio K, Kaya T, Anastasina M. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. Science. 2020;370(6518):856-60. https://doi.org/10.1126/science.abd2985

7. Daly JL, Simonetti B, Klein K, Chen K-E, Williamson MK, Antón-Plágaro C, Shoemark DK, Simón-Gracia L, Bauer M, Hollandi R. Neuropilin-1 is a host factor for SARS-CoV-2 infection. Science. 2020;370(6518):861-5. https://doi.org/10.1126/science.abd3072

8. Li M-Y, Li L, Zhang Y, Wang X-S. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infectious diseases of poverty. 2020;9(02):23-9. Available from: https://mednexus.org/doi/full/10.1186/s40249-020-00662-x

9. Hamming I, Timens W, Bulthuis M, Lely A, Navis Gv, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland. 2004;203(2):631-7. https://doi.org/10.1002/path.1570

10. Wang K, Chen W, Zhou Y-S, Lian J-Q, Zhang Z, Du P, Gong L, Zhang Y, Cui H-Y, Geng J-J. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. biorxiv. 2020. https://doi.org/10.1101/2020.03.14.988345

11. Kasinrerk W, Fiebiger E, Stefanova I, Baumruker T, Knapp W, Stockinger H. Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. The Journal of Immunology. 1992;149(3):847-54. Available from: https://www.jimmunol.org/content/149/3/847.short

12. Koch C, Staffler G, Hüttinger R, Hilgert I, Prager E, Černý J, Steinlein P, Majdic O, Hořejší V, Stockinger H. T cell activation-associated epitopes of CD147 in regulation of the T cell response, and their definition by antibody affinity and antigen density. International Immunology. 1999;11(5):777-86. https://doi.org/10.1093/intimm/11.5.777

13. von Ungern-Sternberg SN, Zernecke A, Seizer P. Extracellular matrix metalloproteinase inducer EMMPRIN (CD147) in cardiovascular disease. International journal of molecular sciences. 2018;19(2):507. https://doi.org/10.3390/ijms19020507

14. Cruzat A, Gonzalez-Andrades M, Mauris J, AbuSamra DB, Chidambaram P, Kenyon KR, Chodosh J, Dohlman CH, Argüeso P. Colocalization of galectin-3 with CD147 is associated with increased gelatinolytic activity in ulcerating human corneas. Investigative ophthalmology & visual science. 2018;59(1):223-30. https://doi.org/10.1167/iovs.17-23196

15. Jin R, Zhong W, Liu S, Li G. CD147 as a key mediator of the spleen inflammatory response in mice after focal cerebral ischemia. Journal of neuroinflammation. 2019;16(1):1-10. https://doi.org/10.1186/s12974-019-1609-y

16. Kato N, Yuzawa Y, Kosugi T, Hobo A, Sato W, Miwa Y, Sakamoto K, Matsuo S, Kadomatsu K. The E-selectin ligand basigin/CD147 is responsible for neutrophil recruitment in renal ischemia/reperfusion. Journal of the American Society of Nephrology. 2009;20(7):1565-76. https://doi.org/10.1681/ASN.2008090957

17. Gwinn WM, Damsker JM, Falahati R, Okwumabua I, Kelly-Welch A, Keegan AD, Vanpouille C, Lee JJ, Dent LA, Leitenberg D. Novel approach to inhibit asthma-mediated lung inflammation using anti-CD147 intervention. The Journal of Immunology. 2006;177(7):4870-9. https://doi.org/10.4049/jimmunol.177.7.4870

18. Zhang J, Ge H, Wang C, Guo TB, He Q, Shao Q, Fan Y. Inhibitory effect of PPAR on the expression of EMMPRIN in macrophages and foam cells. International journal of cardiology. 2007;117(3):373-80. https://doi.org/10.1016/j.ijcard.2006.05.023

19. Chen Z, Mi L, Xu J, Yu J, Wang X, Jiang J, Xing J, Shang P, Qian A, Li Y. Function of HAb18G/CD147 in invasion of host cells by severe acute respiratory syndrome coronavirus. The Journal of infectious diseases. 2005;191(5):755-60. https://doi.org/10.1086/427811

20. Shilts J, Crozier TW, Greenwood EJ, Lehner PJ, Wright GJ. No evidence for basigin/CD147 as a direct SARS-CoV-2 spike binding receptor. Scientific reports. 2021;11(1):1-10. https://doi.org/10.1038/s41598-020-80464-1

21. Wang K, Chen W, Zhang Z, Deng Y, Lian J-Q, Du P, Wei D, Zhang Y, Sun X-X, Gong L. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal transduction and targeted therapy. 2020;5(1):1-10. Available from: https://www.nature.com/articles/s41392-020-00426-x

22. Cary N. Statistical analysis system, User's guide. Statistical. Version 9. SAS Inst Inc USA. 2012.