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Mutation Occurrence in Tor2 Gene in Patients with SARS COV-2 in Association with *H. Influenza* Infection

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SARS COV-2is a very dangerous virus that has led to many deaths. H. Influenzais a bacteria that causes many infections inside the human body, such as pneumonia. In this study, a total of (60)blood samples were taken from patients infected with SARS COV-2shared with H. Influenza infection who attended Ibn-Al-Baladi Hospital/Baghdad city during the period from 15th January to 1st December 2021. Venous blood samples were also taken from (60) healthy individuals as a control group. The results showed that the distribution rate of the SARS COV-2 IgG and H. influenza IgG among the male patients was twice44 (73.3%)more than the distribution rate among female patients. The prevalence of SARS COV-2 IgG and H. influenza IgG was shown to be the highest among the age group (>51) years, followed by the age group (21-30) years. The cases of SARS COV-2and H. influenza infections among the studied patients according to residency were shown to be almost equal among rural and urban residents 30,30 (49.2%,50.8%) respectively. Regarding the relationship between SARS COV-2 IgG and H. influenza IgG and CRP levels, the mean level of CRP in the patients was (73.72±17.05) and in the the controls was (8.71±1.12), while the mean level of H. influenza IgG in the patients was (1.05±0.23) and in the control group was (0.3±0.02), whereas the mean level of SARS COV-2 IgG was (7.00±2.15) in the patients andwas (0.35 ±0.19) in the controls with a highly significant differences (HS). The number and percentage of patients with positive SARS COV-2 and H. influenza IgG was 38(95.0%) who had high levels of GOT up to 65 U/L, while 2(50%) of those patients had GOT >65 U/L, while the Negative infections with SARS COV-2 IgG, H. influenza IgG 17(85.0%) had up 65 U/L and 3(15.0 %) had>65 U/L level (P =0.03).In addition, high levels of GPT, Alkaline phosphates, urea and creatinine were recorded among patients groups when compared with the healthy controls.

Keywords: H. Influenza Infection; Mutation; SARS COV-2; Tor2 gene.

There have been a great demand toSARSCOV-2 tests in the year 2020 in hospitals, health institutions and laboratories around the world, and they have announced their concerns about the lack of materials and laboratory tests for the Corona virus, aspersonal protective equipment were limited, and the speed of detection, as well as contacts, are central to curbing the epidemic spread[1]. Since the appearance of clinical trial results, mass vaccination campaigns toprevent new SARS-CoV-2 infections have been initiated in the countries which can provideadequate vaccine doses. Vaccination campaigns are essential tools, and random clinical tests can identify the population to be vaccinated accurately as they control the logistics, which may not be what is expected to be implemented in health care practice[2]. Out of the seven coronaviruses, four

most frequently cause symptoms of the common cold. Coronaviruses 229E, OC43, NL63, and HKU1 cause about 15 to 30% of cases of common colds. The severe lower respiratory tract infection, such aspneumonia and bronchiolitis can rarely happen, particularlyamong infants, old-aged and immunocompromised individuals[3]. The signs and symptoms of coronavirus infection (COVID-19) may develop 2 to 14 days following exposure. The time after exposure and before symptomappearance is known as the incubation period. Patients can still spread COVID-19 before having symptoms (pre-symptomatic transmission). Common signs and symptoms may includecough, fever and tiredness[4]. SARS 2 and seasonal influenza pathogens share respiratory diseases with the approach of the winter season in the northern hemisphere. They are more common because of the cold winter, and they work side by side in seasonal infections, as well as *Haemophilusinfluenzae* bacteria is common with SARS 2[5]. There is a competition between the coronavirus and respiratory viruses, including influenza, and this interference occurs due to immunity, and it suppresses most of the viruses and activates other viruses,a phenomenon that has been recognized for many decades[6]. Some strains of *H influenzae* don'thave capsules and are termed non-encapsulated *H influenzae* or non-typeable *H influenzae* (NTHi). *Haemophilusinfluenzae* colonization within the respiratory tract, especially the upper respiratory tract, is a risk factor, and this is what compelled doing tests for pharyngeal influenza among healthy populations, and has a potential impact on public health strategy[7]. It is possible to transplant blood or CSF as transplant joint and bone fluid and pericardial fluid and explore standard methods of treatment of Haemophilus influenza if treatment is delayed, and Ocular should be kept at minus 4 to detect cerebrospinal fluid DNA and blood and urine test to detect antigens[8].

MATERIAL AND METHODS

In the current study, a total of (60) blood samples were taken from patients infected with SARS COV-2 shared with *H. Influenza* infection who attended Ibn-Al-Baladi Hospital/Baghdad city during the period from 15th January to 1st December 2021.The anti- SARSCOV-2 IgM antibodies were investigated to detect the acute infection, while anti- SARSCOV-2 IgG antibodies were measured to detect the chronic infection and the healed patients, by using Vides technique.Sandwich ELISA (or sandwich immunoassay) which is the most commonly used ELISA method to detect*H. Influenza*. This format needs two antibody/antigen specific for different epitopes of the antigens/ antibodies. The two antibodies/antigens are usually referred to as matched antibody/antigen pairs. Gene sequenceswere made by using specific primers and were run by a sequencer device. PRIMER PICKING RESULTS FOR NC_004718.3:25268-26092 SARS coronavirus Tor2, complete genome.

LEFT PRIMER	GGGCTTCCAGTTCATTTGCA
RIGHT PRIMER	TGCACTTCCAACAAAGCCAA

Statistical analysis

The statistical analysis was done by the SPSS-20 software program, including t-test and the ($P<0.05$) value was considered statistically significant.

RESULTS

The distribution of the SARS COV-2 IgG and *H. influenza* IgGwas illustrated in table (1). The male rate was twice44 (73.3%) more than femalesaccording to gender, with a significant difference($P<0.05$).

The prevalence of SARS COV-2 IgG and *H. influenza* IgG was shown to be the highest among the age group (>51) years 17(28.3%), followed by the age group (21-30) years 14(23.3%) with no significant difference as shown in table (2).

Table (3) showed that the cases of SARS COV-2and *H. influenza* infections among the studied patients according to residency were shown to be almost equal among rural and urban residents 30,30 (49.2%,50.8%) respectively with no statistical significan difference ($P= 0.04$).

Regarding the relationship between SARS COV-2 IgG and *H. influenza* IgG and CRP levels, the mean level of CRP in the patients was (73.72±17.05) and in the the controls was (8.71±1.12), while the mean level of H. influenza IgG in the patients was (1.05±0.23) and in the control group was (0.3±0.02), whereas the mean level of SARS COV-2 IgG was (7.00±2.15) in the

patients and was (0.35 ±0.19) in the controls with a highly significant differences (P=0.001).

The number and percentage of patients with positive SARS COV-2 and H. influenza IgG was 38(95.0%) who had high levels of GOT up to 65 U/L, while 2(50%) of those patients had GPT >65 U/L, while the Negative infections withSARS COV-2 IgG, *H. influenza* IgG 17(85.0%) had up 65 U/L and 3(15.0 %) had >65 U/L level (P =0.03) as shown in table (5).

Relationship betweenSARS COV-2 IgG, *H.influenza* IgG were explained in table (6). The number and percentage of positive patients 38(95.0%) and high level of GOT toup to 65 U/L, while 2(50%)was detected in patients with>65 U/L, whereasthe number and percentage of negative infections withSARS COV-2 IgG, *H.influenza* IgGwas 17(85.0%) in those with GOT up to 65 U/L and 3(15.0 %) were in those with>65 U/L, (P =0.03).

Relationship betweenSARS COV-2 IgG, *H.influenza* IgG wasshown in table (7).The positive patients 21(55.3%) with high level ofALPamong those withup to 360 U/L and 17(44.7%) were among those with >360 U/L, while the number and percentage ofnegative infections withSARS COV-2 IgG, *H.influenza* IgGwas 2(10.0%) among those with ALP up 360 U/L and 18(90.0 %) was among those with>360 U/L (P =0.01).

Relationship betweenSARS COV-2 IgG, *H.influenza* IgG wasdemonstrated in table (8). The positive patients 16(40.0%) and high level with Ureaup 45 mg/dl and 24(60.0%) was among those with>45 mg/dl, while the negative infections withSARS COV-2 IgG, *H.influenza* IgG 2(10.0%) were up 45 m/dl and 18(90.0 %) were among >360 U/L, P =0.01.

Relationship betweenSARS COV-2 IgG, *H.influenza* IgG wasshown in table (9).The positive patients 14(35.0%) and high level ofcreatininein those with up to 1.3 mg/dl and 26(65.0%) was

Table 1. Prevalence of SARS COV-2 IgG and *H. influenza* IgG according to gender

Gender	Studied group	
	No. of patients	No. of controls
Male	44	30
	73.3%	50%
Female	16	30
	26.7%	50%
Total	60	60
	100.0%	100.0%
P-value	P<0.05 (S)	

Table 2. Prevalence of the SARS COV-2 IgG and *H. influenza* IgG according the age groups

Age / Years	Study group Patient
>10	10
	16.7%
10-20	11
	18.3%
21-30	14
	23.3%
31-40	8
	13.4%
41-50	10
	16.7%
>51	17
	28.3%
P-value	0.2 (NS)

Table 3. Distribution of study group according to residency status

Residency status		Study group		
		Patients	Controls	Total
Rural	N	30	31	61
	%	49.2%	50.8%	100.0%
Urban	N	30	29	59
	%	50.8%	49.2%	100.0%
Total	N	60	60	120
	%	100.0%	100.0%	100.0%
Chi-square	0.04			
P-value	0.81			

among those with>1.3 mg/dl, while the negative infections withSARS COV-2 IgG, *H.influenza* IgG 2(10.0%) was among those with up to 1.3 m/dl and 18(90.0 %) was among those with >1.3 U/L (P =0.01).

In the genetic sequence of the SARS COV-2, the Tor2genetic mutations were found in several positions of gene sequences. Lane (1) reference gen, the change occurrence in Lane 2, T→G, G→A, T→G, G→A, A→G, G→A, A→G,T>A, A→C, A→C, C→A, A→T, T→A, A→T, T→A, Lane 4: A→T, T→A, C→G, A→T, T→C, G→T, C→A and G→A as shown in figure(2).

DISCUSSION

Corona virus and *Haemophilusinfluenzae* may share serious respiratory infections. The distribution of the SARS COV-2 IgG and *H. influenza* IgG illustrated in table (1) showing that the male was twicemore than females according to gender.Based on what was stated by(Renard,

Table 4. Relationshipof SARS COV-2 IgGand *H. influenza* IgG and CRP levels compared to the healthy controls

Parameters	Group	N	Mean	t-test	P-value
CRP	Patient	60	73.72±17.05	8.73	0.001
	Control	60	8.71±1.12		
H.influenza IgG	Patient	60	1.05±0.23	5.53	0.001
	Control	60	0.3±0.02		
SARS COV-2 IgG	Patient	60	7.00±2.15	6.44	0.001
	Control	60	0.35 ±0.19		

Table 5. Relationship between SARS COV-2 IgG, *H.influenza* IgG and GPT levels

SARS COV-2 and *H.influenza* Ig Gcategorical		GPT levels Up65	>65	Total
Positive	N	38	2	40
	%	95.0%	5.0%	100.0%
Negative	N	15	5	20
	%	75.0%	25.0%	100.0%
Total	N	53	7	60
	%	88.3%	11.7%	100.0%

P-value =0.03

Table 6. Relationship betweenSARS COV-2 IgG, *H.influenza* IgG and GOT levels

SARS COV-2 IgG and *H.influenza* IgG categorical		GOT levels Up to 50	>50	Total
Positive	N	38	2	40
	%	95.0%	5.0%	100.0%
Negative	N	17	3	20
	%	85.0%	15.0%	100.0%
Total	N	55	5	60
	%	91.7%	8.3%	100.0%

P-value= 0.3

N.*et al*, 2021), the spread of SARS 2 infection is more in men than in women, and since there is an accompanying *Haemophilusinfluenzae* as well as a compound infection, it has been confirmed that males are more susceptible to these infections, and the reason may be attributed to the fact that men are in more contact with those injuries related to the job or workplace[9]. Prevalence of SARS COV-2 IgG and *H. influenza* IgG according to age groups showed that the age group(>51) contained the most of infections.Mack, D.*et al*., (2021) in his report stated that the injuries of the oldest ages were very prevalent, followed by the ages of their thirties, forties and twenties. This spread is attributed to the physiological conditions of the old ages, especially when there is weak immunity, as well as these ages are more susceptible to diabetes, stress and heart diseases[10]. The equal cases of SARS COV-2and *H. influenza* infections among studied patients according to residency

Table 7. Relationship between Covid-19 IgG, *H.influenza* IgG and ALP levels

Covid-19 IgG and *H.influenza* IgG categorical		ALP levels UP to 360	>360	Total
Positive	N	21	17	38
	%	55.3%	44.7%	100.0%
Negative	N	2	18	20
	%	10.0%	90.0%	100.0%
Total	N	23	35	58
	%	39.7%	60.3%	100.0%

P-value=0.001

Table 8. Relationship betweenSARS COV-2 IgG, *H.influenza* IgGand Urea levels

SARS COV-2 IgG, *H.influenza*IgG categorical		Urea levels Up to 45	>45	Total
Positive	N	16	24	40
	%	40.0%	60.0%	100.0%
Negative	N	2	18	20
	%	10.0%	90.0%	100.0%
Total	N	18	42	60
	%	30.0%	70.0%	100.0%

P-value=0.01

Table 9. Relationship betweenSARS COV-2 IgG, *H.influenza* IgG and creatinine levels

SARS COV-2 IgG, *H.influenza* IgG categorial		Creatinine levels Up to1.3	>1.3	Total
Positive	N	14	26	40
	%	35.0%	65.0%	100.0%
Negative	N	2	18	20
	%	10.0%	90.0%	100.0%
Total	N	16	44	60
	%	26.7%	73.3%	100.0%

P-value= 0.06

betweenruralsand urbans, and this result agreed with (Huang , Q.*et al*, 2021) who reported that the incidence of infections in rural areas is more than in urban areas, and it may be a lack of health awareness in Iraq and the non-existent health care roles, especially in remote areas of Baghdad and provincial centers[11]. There is an increase in CRP levels with SARS 2 infections in association with *Haemophilusinfluenza*. Potempa,A. *et al*, (2021) confirmed that there is a very noticeable increase in the levels of CRP, a protein D, that causes damage to the bodies of the infected, which in turn

Fig. 1. Change occurrence in Lane 2 Rattusnorvegicus, T→G, G→A, T→G, G→A, A→G, G→A, A→G,T>A, A→C, A→C, C→A, A→T, T→A, A→T, T→A, Lane 4: A→T, T→A, C→G, A→T, T→C, G→T, C→A and G→A

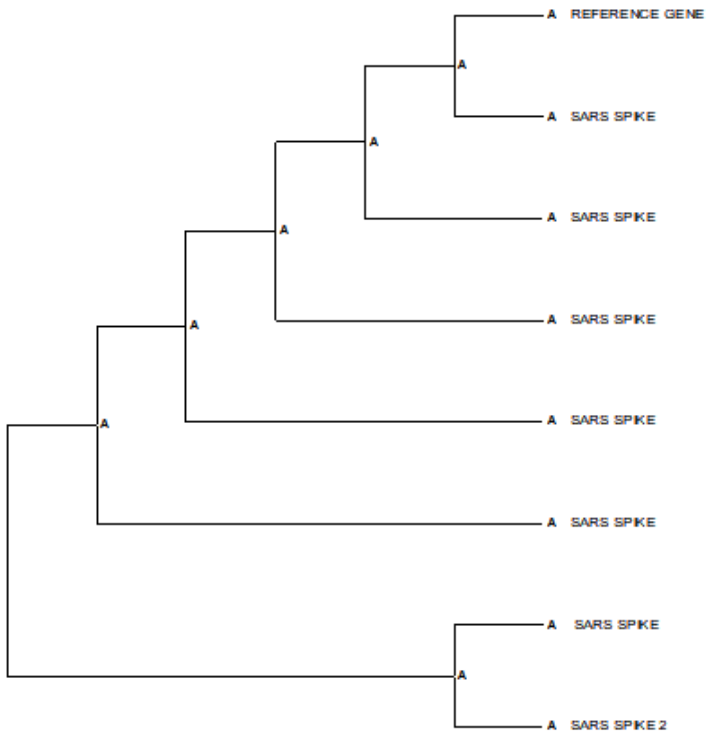

Fig. 2. Phylogenic tree of gene of SARS coronavirus Tor2 in Iraqi patients

increases in these infected people, and since there is a joint infection with *Haemophilusinfluenzae*, there may be a double increase in this protein[12]. Elevation of GPT, GOT, Alkaline phosphatase, urea and creatinine levelsthan their normal levelsin cases associated with infection with SARS-CoV-2 is evident of damage to the liver and kidneys. McGrowder, D. A.*et al*, (2021), in their study stated that there are abnormal levels of liver and kidney functions because infection with this virus invades most of the body's organs, including the liver and kidneys, and thereby, we observed the high level of CRP as an evidence of damage to those organs[13]. In the genetic sequence of the SARS COV-2, Tor2genetic mutations were found in several positions of gene sequence. Chan, A. P.*et al*, (2020) demonstratedgenetic mutation of 10 sites in the genetic chain of the SARS-CoV-2 virus, and these mutations provide the virus with more virulence when it invades human body parts and causes damage to those organs [14].Also these findings are consistent with (So-and-so) who proved that there is mutagenesis in several locations on the gene sequence of the geneBat-CoV RaTG13[15].

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Conflict of interest

Authors declare no conflict of interest.

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REFERENCES

1. Toptan, T. Eckermann, L. and Pfeiffer, A. et al, Evaluation of a SARS-CoV-2 rapid antigen test: Potential to help reduce community spread? *Journal of Clinical Virology,* **135**: 104713 (2021). doi.org/10.1016/j.jcv.2020.104713.
2. Macchia, A. Ferrante, D. and Angeleri, P. et al, Evaluation of a COVID-19 Vaccine Campaign and SARS-CoV-2 Infection and Mortality Among Adults Aged 60 Years And Older in a Middle-Income Country, *JAMA Netw Open*.; **4**(10):e2130800 (2021). doi:10.1001/jamanetworkopen.2021.30800.
3. Tesin, B. L. Coronaviruses and Acute Respiratory Syndromes (MERS and SARS), Lastfull review/revision Sep 2021| Content last modified Oct 2021.
4. CDC Symptoms of COVID-19, Center for disease control and privation Updated Feb. 22, 2021.
5. Stowe, J. Tessier, E. and Zhao, H. et al, Interactions between SARS-CoV-2 and influenza, and the impact of coinfection on disease severity: a test-negative design, *International Journal of Epidemiology,* **50**(4): Pages 1124–1133 (2021) https://doi.org/10.1093/ije/dyab081.
6. Flerlage, T. Boyd, D. F. ad Meliopoulos, V. et al, Influenza virus andSARS-CoV-2: pathogenesis and host responses in the respiratory tract, *Nature Reviews Microbiology*, **19**: 425–441 (2021 s41579-021-00542-7.
7. Yang, P. Zhang, J. & Peng, A. The pharyngeal carriage of Haemophilus influenzae among healthy population in China: a systematic review and meta-analysis, *BMC Infectious Diseases*, **19**:547 (2019). doi.org/10.1186/s12879-019-4195-9.
8. Miller, J. M. Matthew J Binnicker, M. J. and Campbell, Sh. et al, A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology, *Clin Infect Dis*. **67**(6):e1–e94 (2018). doi: 10.1093/cid/ciy381.
9. Renard, N. Soizic Daniel, S. and Cayet, N. et al, Performance Characteristics of the Vidas SARS-CoV-2 IgM and IgG Serological Assays, *J Clin Microbiol.* **59**(4): e02292-20 (2021). doi: 10.1128/JCM.02292-20.
10. Mack, D. Gärtner, B. Ch. and Rössler, A. et al, Prevalence of SARS-CoV-2 IgG antibodies in a large prospective cohort study of elite football players in Germany (May-June 2020): implications for a testing protocol in asymptomatic individuals and estimation of the rate of undetected cases, *Clin. Microbial infect.* **27**(3):473.e1-473.e4 (2021). doi: 10.1016/j.cmi.2020.11.033.
11. Huang , Q. Jackson, S. and Derakhshan, S. et al, Urban-rural differences in COVID-19 exposures and outcomes in the South: A preliminary analysis of South Carolina, *PLOS ONE* | https://doi.org/10.1371/journal.pone.0246548.
12. Potempa, A. Rajab, I. M. and C. Hart, P. C. et al, Insights into the Use of C- Reactive Protein as a Diagnostic Index of Disease Severity in COVID-19 Infections, *Am J Trop Med Hyg*. **103**(2):561–563 (2020). doi: 10.4269/ajtmh.20-0473.
13. McGrowder, D. A. Miller, F. and Cross, M. A. et al, Abnormal Liver Biochemistry Tests and Acute Liver Injury in COVID-19 Patients:

Current Evidence and Potential Pathogenesis, Infectious Diseases; **9**(3):50 (2021). doi:10.3390/diseases9030050.

14. Chan, A. P. Choi Y.W. and Schork, N. J. Conserved Genomic Terminals Of Sars-cov-2 As Co-evolving Functional Elements And Potential Therapeutic Targets, Version bioRxiv. *Preprint* (2020). doi: 10.1101/2020.07.06.190207.

15. Lv, L. Li, G. and Chen, J. et al, Comparative Genomic Analyses Reveal a Specific Mutation Pattern Between Human Coronavirus SARS-CoV-2 and Bat- CoV RaTG13, *Front Microbiol,* **11**: 584717(2020). doi: 10.3389/fmicb.2020.584717.