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Impact of SARS-COV-2 Variants on the Infection Severity among Iraqi Patients

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Abstract

Severe acute respiratory corona viruses (SARS-COVs) are a particular category of RNA viruses that have emerged as a potential danger to the human population, triggering epidemics and pandemics that have resulted in catastrophic human mortality. The SARS-CoV2, responsible for the COVID-19 pandemic that began on December 12, 2019 in Wuhan, China, has been linked to bats. A new SARS-CoV-2 variant appeared in late December 2020. Mutations with variants continued to appear until the time of this study. Thus, this study aimed to provide a local database among Iraqi patients about SARS-COV-2 variants as there have been very few local studies documenting its existence and its relationship with the progression and severity of infection. For this study 234 nasal swabs were collected from COVID-19 positive individuals between March 2021 to March 2022. RNA was extracted and tested by using real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay to confirm infection, and the variants were detected by using a special kit that stratified the characteristic mutations. Results showed the presence of Alpha, Beta or Gamma and Omicron variants in our population at the same time as their global spread at high rates with different severity of cases. It increased in severity during infections with wild type 26/32 (81.25%) and Alpha 82/109 (75.23%) variants but a high incidence of Beta or Gamma 28/38 (73.68%) and Omicron 35/46 (76.09%) variants within mild-moderate infections. Moreover, there was a significant increase in severity in older age groups than younger. Hence, we can conclude that most severe infections with SARS-COV-2 appeared in wild type and during the appearance of Alpha variant which provided a unique database of variants of COVID-19 circulating in the Iraqi population and also assisted in determining the severity of disease. More research is needed on this subject.

Keywords: Variants of SARS- COV-2, cycle threshold, severity, rRT-PCR, TaqPath, COVID-19.

تأثير متغيرات SARS-COV-2 على شدة الإصابة بين المرضى العراقيين

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الخلاصة :

تضم الفيروسات التاجية التنفسية الحادة (SARS−COVs) مجموعة جديدة من فيروسات الحمض النووي الريبي التي تسبب خطر الاوبئة الشديدة للمجتمع والجائحة المسؤولة عن الوفيات. ظهر فيروس SARS−CoV2 المتسبب بجائحة COVID−19 في 12 ديسمبر 2019 في مدينة ووهان في الصين ، كما أثبتت علاقته بالخفافيش. في أواخر ديسمبر 2020 ، ظهر متغير جديد لـ SARS−CoV−2 واستمرت الطفرات مع المتغيرات في الظهور حتى وقت هذه الدراسة. لهذا، هدفت هذه الدراسة السريرية إلى توفير قاعدة بيانات محلية بين المرضى العراقيين حول المتغيرات SARS−COV−2 بسبب ندرة الدراسات المحلية التي توثق وجودها وعلاقتها بتطور الإصابة وشدتها. جمعت 234 مسحة أنف من أفراد مصابين بفيروس COVID−19 إيجابيًا في الفترة من مارس 2021 إلى مارس 2022. استخلص الحمض النووي الريبي واختبر باستخدام اختبار تفاعل البوليميراز المتسلسل (rRT−PCR) اللحظي للنسخ العكسي بغرض تأكيد الإصابة ،كما تم الكشف عن المتغيرات باستخدام عدة تشخيصية متخصصة للكشف عن الطفرات المميزة لكل متغير جديد. أظهرت النتائج وجود متغيرات Alpha و Beta أو Gamma و Omicron مع النمط البري wild type في نفس وقت انتشارها العالمي بمعدلات عالية مع حالات مختلفة من شدة الاصابة. زيادة شدة الإصابة بالنمط البري wild type 32/26 (81.25٪) ومتغيرات ألفاalpha 109/82 (75.23٪)، بينما سجلت نسبة عالية من متغيرات بيتا أو جاما beta or gamma 38/28 (73.68٪) وأوميكرون omicron 46/35 (76.09٪) ضمن الاصابات الخفيفة والمتوسطة. علاوة على ذلك ، سجلت الدراسة زيادة كبيرة في شدة الاصابة لدى المرضى الأكبر سنا. استنتجت الدراسة ، أشد الإصابات بفيروس SARS−COV−2 كانت في النوع البري وأثناء ظهور متغير ألفا ، سيوفرهذا قاعدة بيانات فريدة لمتغيرات COVID−19 المنتشرة في المجتمع العراقي ويساعد في تحديد شدة المرض. هنالك حاجة الى اجراء المزيد من الدراسات حول هذا الموضوع .

1. Introduction

Coronaviruses are the most significant known RNA-enveloped viruses. They have a non-segmented, single-stranded, positive-sense ribonucleic acid (+ssRNA) as their nuclear material [1]. Mild to moderate, severe and critical illnesses in birds, animals and humans are brought on by coronavirus infections. These infections were initially discovered in humans as the common colds causative bacteria. The COVID-19 coronavirus infection, often known as the SARS-CoV2 infection, has already been reported in 210 nations and territories. The World Health Organization has now classified COVID-19 as a pandemic (WHO). More than 394.69 million people have been infected and 5.59 million have died [2]. The first diagnosed virus, that emerged in December 2019, was named wild type This virus carried the conserved sequence in its genome used for diagnosing SARS-CoV. The SARS-CoV-2, like other RNA viruses, is susceptible to genetic evolution as it adapts to its new human hosts through the formation of mutations over time, resulting in the creation of many variations with distinct features from its original strains. In late December 2020, the UK revealed a new SARS-CoV-2 variation of concern, B.1.1.7 lineage, commonly known as Alpha variant or GRY (previously GR/501Y.V1), based on whole-genome sequencing of SARS-CoV-2 [3]. The viral genome of the B.1.1.7 variant had 17 mutations. The spike (S) protein had eight mutations (69-70 deletions, 144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H). N501Y increases spike protein affinity for ACE 2 receptors, boosting viral attachment and subsequent penetration into the host cells [4]. In South Africa's Nelson Mandela Bay in October 2020, a novel SARS-CoV-2 lineage B.1.351 variant known as the Beta variant or GH501Y.V2 with several spike mutations caused the second wave of COVID-19 illnesses [5]. The spike protein of the B.1.351 variant has nine mutations which are found in the RBD and improve the binding affinity for ACE receptors: L18F, D80A, D215G, R246I, K417N, E484K, N501Y, and A701V. This variant has been linked to an increased risk of transmission and a lower ability to be neutralized by monoclonal antibody treatment, convalescent sera and post-vaccination sera. [6]. P.1 is the

third variant of concern, known as Gamma variant or GR/501Y.V3, which was discovered in Brazil in December 2020 and was first detected in the United States in January 2021. The spike protein in the B.1.1.28 variant had ten mutations (L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K and N501Y).

The RBD had three mutations (L18F, K417N, and E484K) [7]. According to the WHO epidemiological bulletin from March 30, 2021, 45 countries picked up this strain. The neutralization of this variation by monoclonal antibody treatments, convalescent sera and post-vaccination sera could be diminished, which is significant [8]. The latest variation of concern, B.1.1.529, also known as the Omicron variant by the WHO, was discovered in South Africa on November 23, 2021, following an increase in the number of COVID-19 patients.. The T91 in the envelope, P13L, E31del, R32del, S33del, R203K, G204R in the nucleocapsid protein, D3G, Q19E, A63T in the matrix, while in the N-terminal domain of the spike appeared N211del/L212I, Y145del, Y144del, Y143del, G142D, T95I, V70del, H69del, A67V. Moreover, the receptor-binding domain of the spike included Y505H, N501Y, Q498R, G496S, Q493R, E484A, T478K, S477N, G446S, N440K, K417N, S375F, S373P, S371L, G339D. Also, D796Y in the fusion peptide of the spike and the heptad repeat 1 of the spikes had L981F, N969K, Q954H, and several additional changes in non-structural proteins and spike protein according to preliminary modeling, and Omicron has a 13-fold boost in viral infectivity. Omicron is projected to experience an extraordinarily disruptive effect because of the spike mutation K417N (observed in the Beta variant) and E484A which increases the likelihood of vaccination breakthroughs [9].

Finally, the current study aimed to provide the local database among Iraqi patients about variants of SARS-COV-2 due to the scarcity of a local studies, thus documenting its existence and relationship to the progression of the disease (clinical data) and severity of infection.

2. Subjects and method

2.1. Case study population

A total of 234 nasal swabs were collected from individuals attending CPHL (Central Public Health Laboratory), Baghdad, Iraq, between March 2021 to March 2022, who, based on their clinical symptoms and the consultant doctor's diagnosis, were thought to be infected with COVID-19. These samples were from 68 females and 166 males between the age of 17-69 years. One hundred thirty-eight cases were in the 11-20 cycle threshold (Ct) range and 96 cases in 21-36 Ct range. According to clinical signs, patients were classified as mild to moderate, severe and clinical infections. The study protocol (University of Baghdad) was authorized by the College of Science Research Ethics Committee (CSEC/0921/0042). To ensure viral vitality throughout transit, the respiratory tract swabs were collected in screw-capped containers containing VTM (viral transport medium). Before RNA extraction, nasal swabs were kept at -70°C. Negative samples and vaccinated individuals were excluded from the study population.

2.2. Molecular Assay

2.2.1. Viral RNA extraction

All specimens were extracted by a special laboratory kit (nucleic acid extraction kit/ magnetic beads method, Zybio manufacturers, China) according to the manufacturer's instructions. The viral RNA was stored at -70°C until SARS-CoV-2 was tested by real-time reverse transcriptase polymerase chain reaction (rRT-PCR).

2.2.2. Reverse Real-Time PCR assay

The yielded RNA was subjected to a real-time reverse transcriptase PCR analysis utilizing a specialized SARS-COV-2 detection kit (Accupower® SARS-COV-2 multiplex real time RT-PCR kit, Bioneer, Korea). Real-time PCR thermal cycler was used with the plate (ABI 7500 fast) with the amplification that follows: reverse transcription activation step at 50℃ for 20 minutes, pre-denaturation at 95℃ for 5 minutes, followed by five cycles of touch down at 95℃ for 35seconds, then 95℃ for 5 seconds denaturation and 58℃ for 30 seconds annealing for 40 cycles. The cases that were less than 37 cycle numbers were taken as positive diagnostic.

2.2.3. SARS-CoV2 variants detection

All 234 samples were positive for SARS-CoV-2. Tests were performed for detecting SARS-COV-2 variants using Accu-power® SARS-CoV-2 variants ID 1 kit (Bioneer, Korea). 10 µl of master mix was added in each well and 10 µl of specimens or controls. Amplification was performed on the plate using the real-time PCR thermal cycler (ABI 7500 fast): activation RT step at 50℃ for 15 minutes, pre-denaturation at 95℃ for 5 minutes, followed by 45 cycles at 95℃ and 57℃ for 35 seconds. This method was used to diagnose wild type, Alpha, Beta and Gamma variants stratified to specific mutations designed in the kit. Wild-type virus was diagnosed by carrying only the conserved sequence SARS-CoV in its genome without carrying any other mutations designed in the kit. Alpha variant was diagnosed by carrying 69/70 DEL, N501Y, P681H, and E484K mutations in addition to SARS-CoV conserved sequence and negative for S gene. Beta or Gamma variants were diagnosed by carrying N501Y, P681H, K417N/T mutations, in addition to SARS-CoV conserved sequence and being positive for detecting S gene.

2.2.4. Detection of Omicron variant

Diagnosis of Omicron was performed by using the TaqPath (TaqPATH COVID-19 CE-IVD RT-PCR Kit, thermos fisher, Germany) COVID-19 PCR test. Infections were classified as SGTF (S Gene Target Failure Assay) when a patient's TaqPath COVID-19 PCR test was positive, and the ORF1ab or nucleocapsid gene targets had a cycle threshold of 36 or fewer, but the S gene wasn't detectable [10]. To prevent identifying infections for which the S gene was not found (due to low viral load) as SGTF, were confined to a cycle threshold of 36 or fewer (i.e., high cycle threshold values). When a patient tested positive for the TaqPath COVID-19 PCR test with a cycle threshold of 36 or fewer for either the ORF1ab or nucleocapsid gene targets and had a detectable S gene target, the infection was categorized as non-SGTF [11]. Omicron cases were conformed using Accu-power® SARS-COV-2 variants ID kit (Bioneer, Korea). Positive result were observed for the mutations 69-70 Del, N501Y from the Alpha variant and K417N mutation from the Beta variant.

2.3. Statistical Analysis

Frequencies and percentages were managed and analyzed using the statistical package versions of GraphPad Prism 8.0.0 and IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY). Continuous variables were first checked with a normality test to describe the median with an interquartile range for not being normally distributed. Mann-Whitney U and Kruskal-Wallis tests were used to assess significant differences between medians. The categorical variables were presented as numbers and percentages and were analyzed by the two-tailed Fisher exact or Pearson Chi-square tests to compare frequencies. A probability (p) value < 0.05 was taken as statistically significant.

3. Results and Discussion

3.1. Demographic of SARS-COV-2 patients

A total of 234 patients were included in this study. They were divided in two groups as age median (54 [IQR: 51 - 59] years; $p < 0.0001$) and (38 [IQR: 30.5 - 42] years; $p < 0.0001$), as can be seen in Table 1. The information in the table shows that older ages had a higher chance of contracting COVID-19 and developed more severe illness. These findings supported other research findings of a favorable association between age and COVID-19 infection progress that older people are at a higher risk of developing more severe illness than younger ones [12]. This condition was due to the changes in body's immune response to infection, thus affecting immune cells' response and mediators in peripheral tissues. These changes determine not only the susceptibility to infection but also disease progression (severity) and clinical risks in the future [13]. In the case of sex, males (166 vs. 70.94%) outnumbered females (68 vs. 29.06%) in disease Numerous studies have revealed a strong link between gender and the severity of the condition, with males being more vulnerable than females as they develop more severe and critical illness [14]. The Ct value between 11- 20 cycles had more frequency 138 (58.97%) than that of Ct value between 21-36 which had a frequency of 69 (41.03%). The results obtained in the current study agree with many other clinical studies that have been approved. A low Ct value with an elevated concentration of viral load typically correlated with higher infection severity [15]. In case of disease severity, most patients included in the study suffered from severe 88 (37.6%) and critical 50 (21.37%) infections respectively. Most severe and critical cases fell in the infection with wild type and Alpha variant, while most Beta or Gamma and Omicron variants infections fell within mild to moderate. Finally, in the case of infection with SARS-COV-2 variants, Alpha variant had the most elevated infection percentage 109 (46.6%) due to time of sample collection (March 2021) as in the time of Alpha variant dominance over wild type, followed by Beta or Gamma variants 47 (20.1%), then Omicron variant 46 (19.6%) and wild type 32 (13.7%).

Table 1: Baseline characteristics of patients with SARS-CoV-2 infection

Characters		SARS-CoV-2 Cases: n = 234	p-value
Age: Years	≤ 45	38 (30.5 – 42)	**< 0.0001**
	> 45	54 (51 – 59)	
Sex	Male	166 (70.94)	**< 0.001**
	Female	68 (29.06)	
Ct Threshold Values	11 - 20	138 (58.97)	**< 0.01**
	21 - 36	96 (41.03)	
Severity Group	Mild-Moderate	96 (41.03)	**< 0.001**
	Severe	88 (37.6)	
	*Critical	50 (21.37)	
SARS-CoV-2 Variants	Wild type	32 (13.7)	**< 0.001**
	Alpha	109 (46.6)	
	Beta or Gamma	47 (20.1)	
	Omicron	46 (19.6)	

Values of age are given as median with interquartile range (discontinues variables) or number and percentage (categorical variables);*: included six of death cases; *p*: probability of Mann-Whitney U test (to compare discontinues variables), two-tailed Fisher exact test or Pearson Chi-square test (to compare categorical variables).

Table 2 shows that frequencies of SARS-CoV-2 infection and variants stratified with age, gender, severity and Ct values revealed a statistically significant association between age and

the variants. Younger age patients (≤ 45) were more likely to be infected with Alpha variant than older people (p <0.05) as compared with wild type. At the time of this study's sample collection in March 2021, the Alpha variant started to overcome wild type infecting more people worldwide. Furthermore, the Ct threshold values showed that infection with wild type had a significant relation with Ct threshold (p <0.05). The reason being that patients infected with wild type were more likely to develop a severe and critical illness with a higher viral load which is inversely related with Ct threshold values that lead to an increase in severity during infection with wild type 26/32 (81.25%), even in the lower number of patients with three death cases (50% of deaths). During infection with Alpha variant, 82/109 (75.23%) of patients developed severe and critical illness and lower Ct values with higher viral load, but an increased incidence of beta or gamma 28/38 (73.68%) and omicron 35/46 (76.09%) variants fell within mild-moderate infections, higher CT values and lower viral load. Although PCR variant kit couldn't discriminate between Beta and Gamma variants, at the time of data collection (March 2021), Beta variant was the dominant infection worldwide. Gamma variants were still undiagnosed. Beta variants could still cause severe and critical illness with three death cases. 50% patients had other comorbidities (diabetes, hypertension, heart diseases, renal failure, etc.) in severe and instances of death. In late March 2021, Gamma variant was diagnosed in 45 countries worldwide, transmitting faster than the Beta variant and infecting more people and causing a high incidence of mild and moderate disease, elevating the ratio of mild illness, and making it significant. In contrast, Omicron variant showed non-significant relation with age and gender, the Ct value, and the severity of infection [16].

Table 2: Frequencies of SARS-CoV-2 infection and variants stratified to patient ages,sex, severity and Ct threshold values.

Characters; n = 234		**Wild type**	**Variant of SARS-CoV-2**			**Total**	***P*-value**
			Alpha (B.1.1.7)	Beta (B.1.351) or Gamma (P.1)	Omicron (B.1.1.529)		
Age: Year	≤ 45	15	66	21	23	125	< **0.001**
	> 45	17	43	26	23	109	< **0.01**
***p*-value**		*p* =0.72	< **0.05**	*p* =0.47	--		
Sex	Male	23	84	33	26	166	< **0.001**
	Female	9	25	14	20	68	< **0.05**
***p*-value**		< **0.05**	< **0.001**	< **0.01**	*p* =0.38		
Ct Threshold Values	11 – 20	**26**	**65**	25	22	138	< **0.001**
	21 - 36	6	44	22	24	96	< **0.001**
***p*-value**		< **0.001**	< **0.05**	*p* =0.67	*p* =0.77		
Severity Group	Mild-Moderate	6	27	28	**35**	96	< **0.01**
	Severe	**15**	**51**	11	11	88	< **0.001**
	Critical (dead)	**8 (3)**	**31** (0)	5 **(3)**	0 (0)	44 (6)	< **0.01**
***p*-value**		*p* =0.13	< **0.05**	< **0.01**	*p* =0.18		

; *p*: probability of two-tailed Fisher exact test or Pearson Chi-square test (to compare between frequency in categorical variables).

Figure 1 shows the results of rRT-PCR (Ct threshold value) stratified to frequency of age groups and severity (mild-moderate, severe and critical) of COVID-19 patients. However, case severity non-significantly increased with Ct value (p = 0.53) in the results obtained from the

study. Still, the most severe and critical cases, 86/98 patients (87.76 %) included in the study, fell within the Ct value range of (10-20). This finding led us to conclude that lower Ct value cases had higher viral load and more severe or critical illness. These results also supported the majority of prior research that found an adverse relationship between greater viral loads related to Ct values and severity of illness [17]. While lower viral load with higher Ct values (46/57) patients fell within the range of 21-36 and developed less severe infection. In addition, patients age non-significantly increased with Ct values (p= 0.43) when 138 patients fell within 10-20 Ct value range. 96 patients fell within 20-36 Ct value range. The results approved that Ct value range had no relation to age groups.

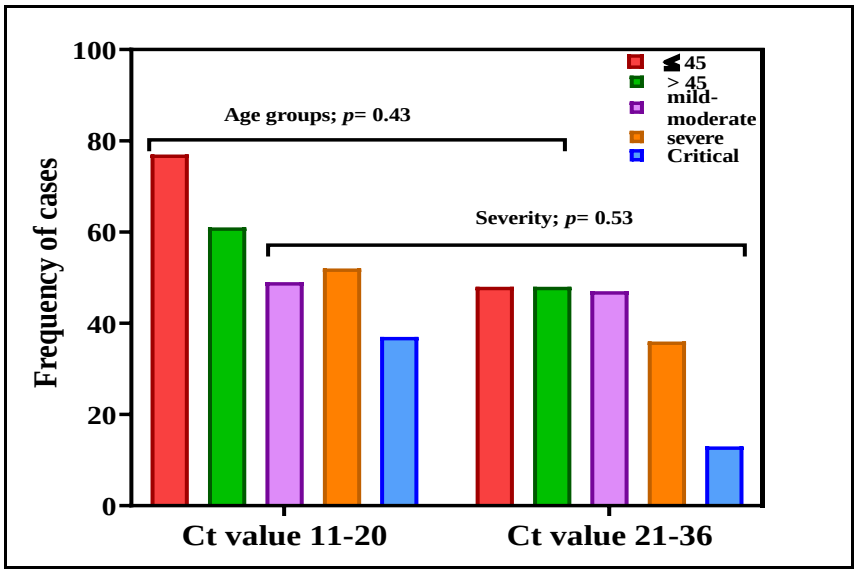

Figure 1: Frequency of age groups and severity (mild-moderate, severe and critical) of COVID-19 patients stratified to Ct threshold values. *p*: Two-tailed Fisher exact Chi-square test probability.

3.2. Age distribution to sex and disease severity

Figure 2 reveals that the results obtained in this study show that median age (in interquartile range) in COVID-19 patients was non-significant between males and females (p=0.98). In contrast, the age median had a highly significant relationship with severity (p <0.0001). The highest median of age 53 (42.8-59) was recorded in critical disease severity, followed by median age of 46 (35.3-55) in severe cases and finally median age of 42 (36-48) in mild to moderate illness. The results showed that older people were more likely to develop critical and severe diseases than younger patients. Other studies revealed that patients over 60 years were more likely than younger patients to have severe and life-threatening COVID-19 disorders [18].

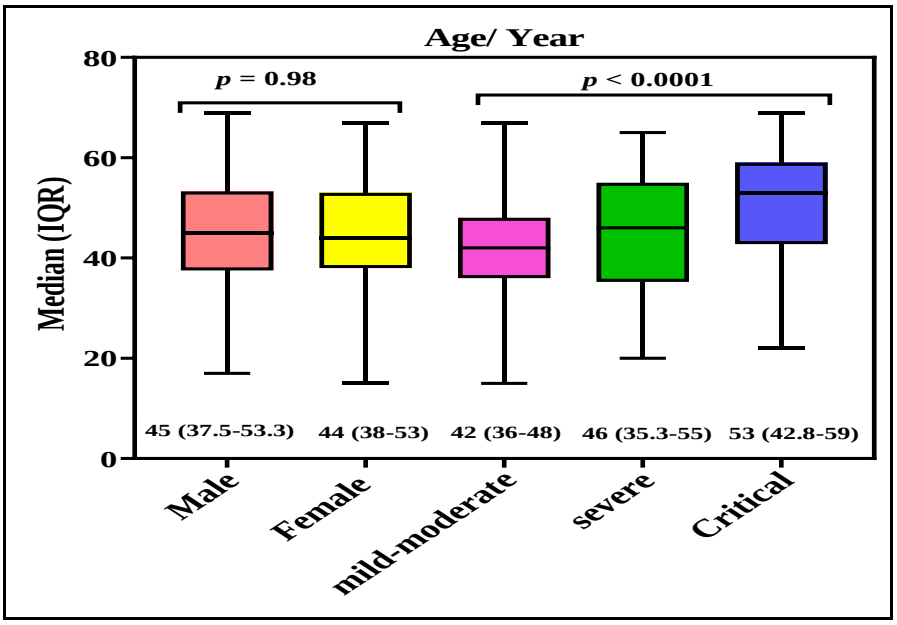

Figure 2: Box plot of age (median and interquartile range) in COVID-19 patients according to gender and disease severity. *p*: Kruskal-Wallis test and Mann-Whitney *U* test probability were used to evaluate significant variations in medians

3.3. SARS-CoV-2 variants correlation with Age and Ct value

Figure 3 shows that age median (in the interquartile range) had non-significant relation with the variants of COVID-19 (P=0.053). This result approved that age had no relation to infection with different variants of SARS-COV-2. The Ct threshold value had a significant relation with infection with different variants of COVID-19 (p <0.01), where the lowest Ct value (17.1) appeared in infection with wild type, meaning that the highest viral load and the most severe disease was followed by (17.2) during infection with Alpha variant that also had high viral load and developed a more severe and critical illness. In contrast, Beta (20.5) and Omicron (22.1) variants showed higher Ct values with lower viral load and developed more moderate and milder illnesses. While Beta (20.5) and Omicron (22.1) variants showed higher Ct values with lower viral load and developed more moderate and milder illness.

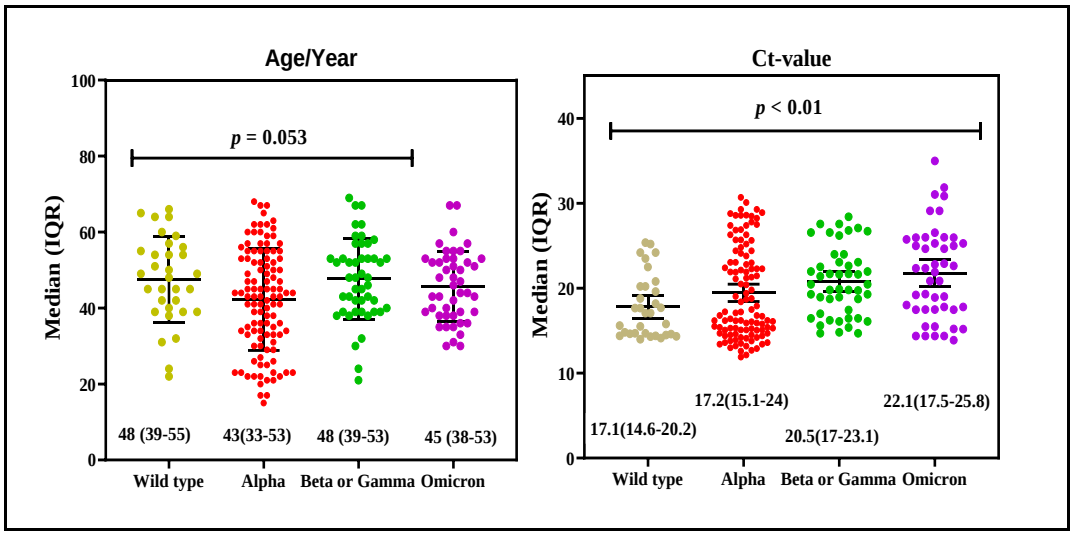

Figure 3: Scatter plot of age and Ct value (median and interquartile range) in COVID-19 patients according to SARS-CoV-2 variants. *p*: Kruskal-Wallis test probability was used to evaluate major variations in medians

Conclusions

Our data indicates that older people are more susceptible to SARS-COV-2 infection, particularly those with comorbidities (hypertension and diabetes). Additionally, individuals with pre-existing illnesses and older ages had more fatality ratio. Also, a lower Ct value during infection with wild-type and Alpha variants indicated higher viral load and more severe and critical conditions. This could be a potential prognostic factor, confirming the importance of developing a local database of variants circulating in the Iraqi population and in assessing case severity and disease progression. Further research is recommended.

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Conflict of Interest

There were no conflicts of interest as declared by the authors.

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