Bionatura Journal
Ibero-American Journal of Biotechnology and Life Sciences
Article

Detection of SARS-CoV-2 variants distributed from March 2020 to May 2022 and their effect on the infection severity in Iraqi population

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Available from. http://dx.doi.org/10.21931/BJ/2024.01.01.41

ABSTRACT

New SARS-CoV-2 variants appeared in late December 2020 as Mutations accumulated in the original virus. This study aimed to provide a local database about variants of COVID-19 circulating in the Iraqi population from 2020 to 2022 and the time of emergence of new strains each month since very few local studies have documented its existence in the country. Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assays were employed to 319 collected and analyzed nasal swabs to determine whether an infection had occurred. A sophisticated diagnostic kit that sorted the distinctive mutations was implemented to evaluate the variants. Results showed Younger patients were more likely to be infected with the Alpha variant (66 patients) than older people (43 patients).

Additionally, patients with wild-type infestations had more robust viral load and lower Ct threshold values, culminating in an increase in severity during infection with wild-type virus 26/32 (81.250). Meanwhile, 65/109(59.63%) of patients infected with the Alpha variant developed severe and critical illness and 51/84(60.71%)were infected with Delta or Delta plus variants. In conclusion, the Alpha variant had the highest infection percentage of 109(46.6%), followed by Delta or Delta plus variant 84(26.33%), Beta or Gamma variants 47(20.1%), Omicron variant 46(19.6%), and finally wild-type virus of 32(13.7%). February 2020 witnessed a preliminary finding of the wild-type, while the Alpha variant emerged in December 2020, Beta/Gamma variances were recognized in December 2020, Delta/Delta plus variances began in April 2021, and the Omicron variant debuted in March 2022.

Keywords: SARS-CoV-2**,** Mutation, rRT-PCR, Coronavirus disease 2019, TaqPath, cycle threshold (Ct) value

INTRODUCTION

Since WHO pronounced SARS-CoV-2 an international epidemic on March 11, 2020, it has continued to bring about catastrophe[1]. Like other RNA viruses, SARS-CoV-2 is susceptible to genetic evolution as it accumulates modifications over time to adapt to its new human hosts. As a result, several variants that could be dissimilar from its ancestors' strains emerge. On the backbone of whole-genome sequencing of samples from patients who tested positive for SARS-CoV-2 in the UK in late December 2020, a novel SARS-CoV-2 variant of concern, the Alpha variant, was discovered[2]. The Alpha variant has been recognized as a consequence of genome sequencing. A commercial test technique is becoming increasingly prevalent and is characterized by the absence of S gene (S-gene target failure, SGTF) PCR results. The viral genome of this variant exhibits a total of 17 mutations. The spike (S) protein is impacted by eight mutations: N501Y, A570D, P681H, T716I, S982A, and D1118H. Furthermore, there were additionally 144 deletions and 69–70 deletions. The spike protein's affinity for ACE 2 receptors is enhanced by N501Y, which promotes viral attachment and subsequent host cell penetration[3]. Tegally and his coworkers discovered an uncommon SARS-CoV-2 lineage, the Beta structure, in Nelson Mandela Bay, South Africa, in October 2020. This discovery led to the second wave of COVID-19 cases[4]. Nine changes have been made to the spike protein in this variant, three of which—K417N, E484K, and N501Y—are positioned in the RBD and improve the protein's affinity for ACE receptors. Spike protein includes three additional mutations, including L18F, D80A, D215G, R246I, and K417N[5]. The third concern variant, the P.1 and Gamma variant was first made public in the US in January 2021[6]. The spike protein is made up of the following ten mutations in the variant above: L18F, T20N, P26S, D138Y, R190S, H655Y, T1027I V1176, K417T, E484K, and N501Y. According to WHO epidemiological data from March 30, 2021, this strain has been found in 45 countries. The devastating second wave of COVID-19 infections that struck India in April 2021 was caused by the fourth strain of concern, the Delta variation. However, the WHO classified this type as a variant of concern (VOC) in May 2021 because of its rapid global expansion. Ten mutations, including T19R, G142D, 156del, 157del, R158G, L452R, T478K, D614G, P681R, and D950N7, have been found in the spike protein of this variant[7]. The Delta Plus version first gained popularity in India, but recent research has shown that other nations are experiencing quicker expansion. The K417N mutation in the spike protein is unique to the strain Delta Plus. The first sequence of this kind, according to media accounts, was found in March 2021 in Europe[7]. On November 23, 2021, the Omicron variant of COVID-19, the sixth COVID-19 variant, was found in South Africa. Approximately 30 mutations are present in total, including T91 in the envelope, P13L, E31del, R32del, S33del, R203K, G204R in the nucleocapsid protein, D3G, Q19E, A63T in the matrix, N211del/L212I, Y145del, Y144del, Y143del, G142D, T95I, V70del, H69del[8] . The Kappa variant, which was first discovered in India in December 2021 and is an interesting variant, has been categorized by the WHO and the CDC as having the T95I, G142D, E154K, L452R, E484Q, D614G, P681R, and Q1071H mutations[7]. Thus, this study aimed to provide, in part, a local database

about variants of COVID-19 circulating in the Iraqi population from 2020 to 2022 and the time of emergence of new strains during each month since there have been very few local studies documenting its existence in the country and its relationship with the progression and severity of infection.

MATERIALS AND METHODS

Data source

From March 2020 to May 2022, 319 nostril swabs have been gathered in Baghdad, Iraq. To diagnose COVID-19 infection, viral RNA was separated from the specimens and sent to the Central Public Health Laboratory (CPHL) in Baghdad while being carried chilled in ice. Collected specimens were from patients aged 15 – 69 years old with **a** median of 43 (38 – 53) and were 226 males and 93 females with different social levels and grouped into two age groups: ≤ 45= 174 with a median of 39 (32 – 42) and > 45= 145 with a median of 53 (52 – 59).

Viral nucleic acid Extraction

All samples were extracted using a specialized laboratory kit (AddPrep Viral Nucleic Acid Extraction Kit, Add bio, Korea) following the manufacturer's instructions. The viral RNA was kept at -70 °C before the SARS-CoV-2 real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test.

Real-Time PCR assay

The extracted RNA was analyzed for SARS-CoV-2 infection employing real-time reverse transcriptase PCR utilizing a particular SARS-CoV-2 detection kit (AccuPower® SARS-CoV-2 Multiplex RT-PCR Kit, Bioneer, Korea). Following the reverse transcription activation step at 50°C for 20 minutes, pre-denaturation at 95°C for 5 minutes, five cycles of touch down at 95°C for 35 seconds, 95°C for 5 seconds of denaturation, and 58°C for 30 seconds of annealing for 40 cycles, the plate was examined with an ABI 7500 fast device with a real-time PCR thermal cycler. Cases with cycle numbers beneath 36 have been categorized as positive diagnostic cases.

SARS-CoV-2 variants detection

All 319 samples were positive for SARS-CoV-2. The SARS-CoV-2 variants have been determined via tests using the Accu-power® SARS-CoV-2 variants ID 1 kit (Bioneer, Korea). The SARS-CoV-2 variants were identified during experiments. 10 µl of the master mix and 10 µl of the samples or controls were given to each well. A real-time PCR thermal cycler (ABI 7500 fast) has been used to perform the activation RT stage on the plate for 15 minutes, followed by 5 minutes of pre-denaturation at 95°C and 45 cycles at 95°C and 57°C for 35 seconds each. This technique identified the wild type, Alpha, Beta, and Gamma variants based on specific mutations specified in the kit. The SARS-CoV conserved sequence was the only mutation the wild-type virus had in its genome; other mutations from the kit were absent. The 69/70 DEL, N501Y, P681H, and E484K mutations in the SARS-CoV-2, The N501Y, P681H, K417N/T mutations, the SARS-CoV-2 conserved sequence, and a positive S gene test result were utilized to diagnose the Alpha variant. At the same time, they were also used to diagnose the Beta and Gamma versions. The Omicron variation was diagnosed using the TaqPath COVID-19 PCR test (TaqPath COVID-19 CE-IVD RT-PCR Kit, Thermo Fisher, Germany). 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 lower, but the S gene was not detectable[9]. To prevent identifying infections for which the S gene was not found (due to low viral load), infections with a cycle threshold of 36 or fewer (high

cycle threshold values) were omitted from the analysis. When a patient tested positive for the TaqPath COVID-19 PCR test with a cycle threshold of 36 or lower for either the ORF1ab or nucleocapsid gene targets and had a detectable S gene target, the infection was categorized as non-SGTF[10]. Omicron variant infected cases were confirmed using Accu-power® SARS-CoV-2 variants ID kit (Bioneer, Korea). Positive results were observed for the mutations 69-70 Del and N501Y from the Alpha variant and K417N mutation from the Beta variant.

Statistical analysis

IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) was applied to statistical analysis. Version 8.0.0 of GraphPad Prism was utilized as well. When the data is not distributed generally for continuous variables, the normality test defines the median with an interquartile range. The significance of discrepancies between the medians is further examined using the Mann-Whitney U and Kruskal-Wallis tests. When categorical variables, which are presented as numbers and percentages, are compared for frequency, the two-tailed Fisher exact test or Pearson Chi-square test is utilized. A probability (p) value of 0.05 was used to define statistical significance.

RESULTS

Baseline characters of SARS-CoV-2 patients

A total of 319 patients were included in this study. They were divided into two age groups: ≤45= 174 with a median of 39 (32 – 42) and > 45= 145 with a median of 53 (52 – 59), as can be seen in Figure 1. According to the statistics in Figure 1, there was a highly significant link between age and SARS-CoV-2 infection ($p < 0.0001$), suggesting older people had a greater chance of catching COVID-19 and experiencing more severe sickness. The disease affected more men (226 vs. 70.85%) than women (93 vs. 29.15%) in terms of sex ($p < 0.001$). In comparison to the Ct value between 11-20 cycles, which had a higher frequency of 188 (58.93%) ($p < 0.05$). Then, that of Ct value between 21-36, which had a frequency of 131 (41.07%) were ($p < 0.05$). In terms of disease severity, the majority of the patients included in the study had severe infections in 125 (39.18%) and critical infections in 81 (25.39%), which, respectively, had six fatal cases. 113 (35.42%) had mild to moderate infections ; contrast was ($p < 0.01$).

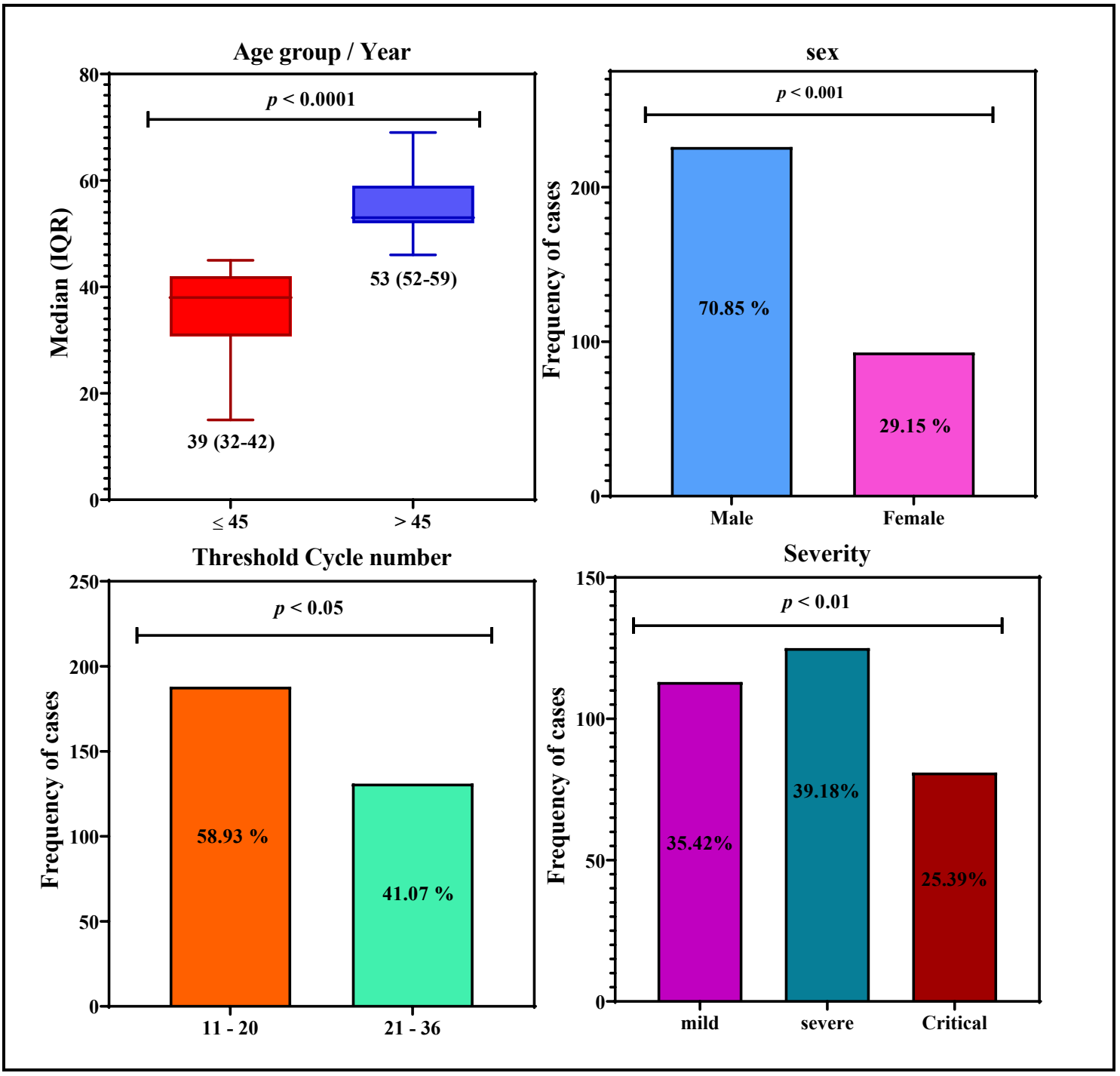

Figure 1. Baseline characters of SARS-CoV-2 patients; p: probability of Mann-Whitney U test (to compare discontinued variables), two-tailed Pearson Chi-square test (to compare categorical variables).

Baseline characters of SARS-CoV-2 infections stratified with variants of COVID-19

Table 1 shows that infection frequencies with COVID-19 and its variants stratified with age, gender, severity and Ct values revealed a statistically significant correlation between age and the variants. Younger patients (≤ 45) were more likely to be infected with the Alpha variant (66 patients) than older people (43 patients) were ($p < 0.05$) as compared with wild type. Furthermore, the Ct threshold values showed that wild-type infection significantly correlated with Ct threshold ($p < 0.001$). The reason is 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 to 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 the Alpha variant, 65/109 (59.63%) of patients developed severe and critical illness and lower Ct values with higher viral load were ($p < 0.05$), and 51/84 (60.71%) were infected with Delta or Delta plus variants were ($p=0.05$). Although the PCR variant kit couldn't discriminate between Beta and Gamma variants and Delta or Delta plus variants, at the time of data collection (March 2021), the Beta variant was the dominant infectious variant worldwide. Gamma variants were still undiagnosed. Beta variants could still cause severe and critical illness, with three death cases (50%) for patients who may have had other comorbidities (diabetes, hypertension, heart diseases, renal failure, etc.) in severe instances of death. The gamma variant, which spreads more swiftly than the Beta variant, infects more individuals and causes a high incidence of mild and moderate disease, boosting the ratio of mild illness and making it noteworthy, it was discovered in late March 2021 in 45 nations throughout the entire globe.

In contrast, the Omicron variant did not significantly correlate with gender, age, Ct value, or infection severity. However, In comparison to the wild type of the virus, which was present in 15 patients with severe infections and 8 patients with critical infections, the Alpha variant was present in 51 patients with severe infections, and 31 patients with critical infections, and the Delta or Delta plus variant was present in 37 patients with severe infections and 31 patients with critical infections. At the same time, most Omicron and Beta or Gamma variant illnesses were considered mild to severe. At the time of sample collection (March 2021), when SARS-CoV-2 variants predominated over wild type, the Alpha variant had the highest infection percentage of 109 (46.6%) followed by Delta or Delta plus variant 84 (26.33%), Beta or Gamma variants 47 (20.1%), Omicron variant 46 (19.6%), and wild type virus of 32 (13.7%).

Characteristic; 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)	Delta or Delta Plus	Kappa	Omicron (B.1.1.529)		
Age; year	≤ 45	15	**66**	21	48	1	23	174	**< 0.001**
	> 45	17	43	26	36	0	23	145	**< 0.01**
***p*-value**		*p* =0.72	**< 0.05**	*p* =0.47	*p* = 0.19	--	--		
Sex	Male	23	84	33	60	0	26	226	**< 0.001**
	Female	9	25	14	24	1	20	93	**< 0.05**
***p*-value**		**< 0.05**	**< 0.001**	**< 0.01**	**< 0.001**	--	*p* =0.38		
Ct threshold values	11 - 20	**26**	**65**	25	**51**	1	22	188	**< 0.001**
	21 - 36	6	44	22	33	0	24	131	**< 0.001**
***p*-value**		**< 0.001**	**< 0.05**	*p* =0.67	*p* =0.05		*p* =0.77		
Severity group	Mild-moderate	6	27	28	16	**1**	**35**	113	**< 0.01**
	severe	**15**	**51**	11	**37**	0	11	125	**< 0.001**
	Critical (dead)	**8 (3)**	**31** (0)	5 **(3)**	**31 (0)**	0	0 (0)	75 (6)	**< 0.01**
***p*-value**		*p* =0.13	**< 0.05**	**< 0.01**	**< 0.05**	--	**< 0.01**		

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

Table 1. Frequencies of SARS-CoV-2 infection and variants stratified to patient ages, gender, severity and Ct threshold values.

Distribution of SARS-CoV-2 infection by months from Mar 2020 to May 2022

Figure 2 illustrates the appearance of various SARS-CoV-2 variants during 2020, 2021, and 2022 years, respectively. Late February of 2020 witnessed a preliminary finding of wild type in Iraq, which persisted throughout December of the same year. The inaugural Alpha variant emerged in December 2020, continued throughout 2021, and declined in May 2022. Beta or Gamma variances were initially recognized in December 2020 and eventually diagnosed in April 2022. The Delta or Delta plus variances diagnosis began in April 2021 and continued throughout April 2022. The official identification of the Omicron variant debuted in March 2022, and new sub-lineages continue to appear.

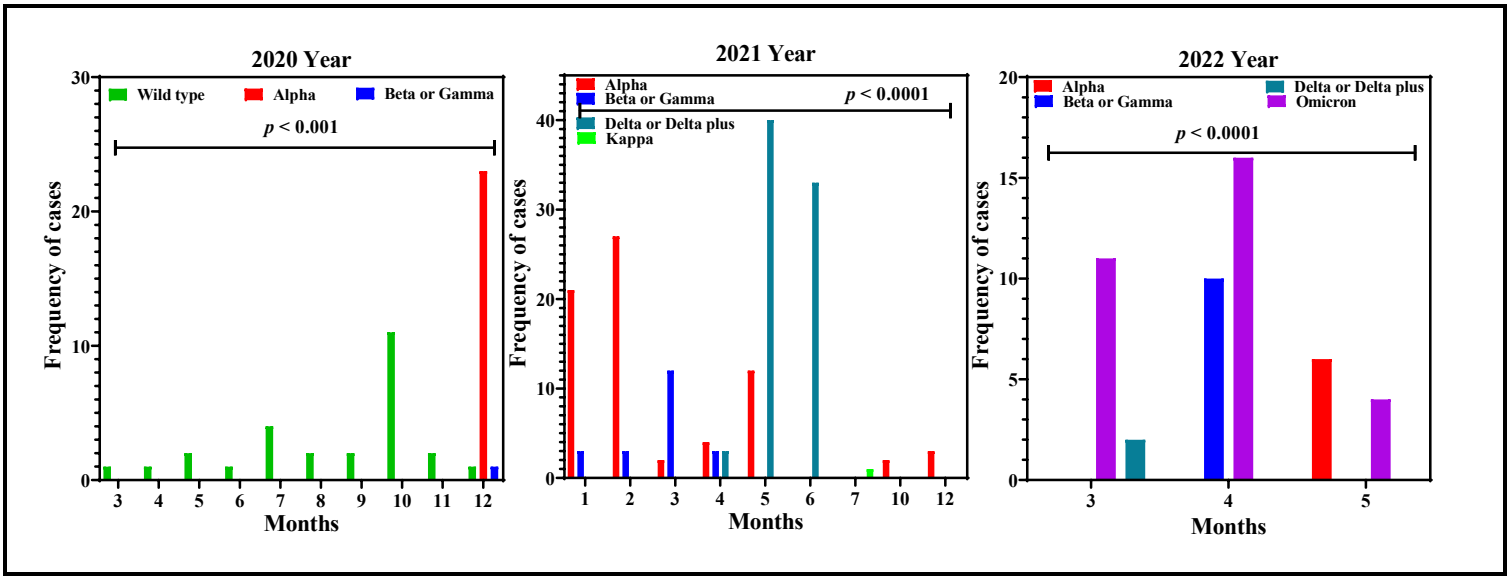

Figure 2. Distribution of SARS-CoV-2 infection by months from March 2020 to May 2022; p: probability of two-tailed Pearson Chi-square test (to compare categorical variables).

DISCUSSION

The severity of the infection and the development of the illness were significantly influenced by the age of the research participants, with older people >45 years being more impacted than younger participants. This finding is consistent with recent studies, showing a causal relationship between aging and COVID-19 infection[11]. Possible explanations include co-morbid illnesses and weakening immune systems, which increase an older person's susceptibility to numerous infectious agents (including SARS-CoV-2) with the infection's rapid progression and worst-case scenario. Given that the number of people with weakened immune systems in a community correlates with age structure, age appears to be a substantial risk factor for COVID-19 prior comorbidities. Patients in various age groups have different risk factors, as shown by the differences in the kinds of comorbidities that already exist and the physiological changes that occur as people age. Age had no impact on different COVID-19 variant infections. According to other studies, older patients (>60 years) are more prone than younger patients to exhibit severe and life-threatening COVID-19 symptoms[12]. The current study's findings showed that gender significantly influenced the severity of the infection and the course of the condition; males were more likely to get an infection and had more severe consequences. Though it is yet too early to determine the precise explanation, the X chromosome, sex-based immunological variations brought on by sex hormone (sex-specific steroids), and

higher angiotensin-converting enzyme-2 (ACE 2) production in males than females are all plausible causes of the gender gap. The enzyme ACE2 is a component of the renin-angiotensin-aldosterone system (RAAS), a hormonal system that regulates blood pressure, tissue perfusion, and the balance of the extracellular environment. Men are generally more susceptible than women in that they encounter a greater likelihood of severe and critical sickness, in line with numerous studies, and there is a significant association between gender and the severity of the illness[13]. The current investigation results agree that numerous previous clinical trials were granted authorization. A low Ct value with a high viral load concentration typically suggests an increasingly grave infection[14]. Despite growing population immunity, the current SARS-CoV-2 variants contain several changes that allow them to continue reproducing and spreading. These mutations are among a group of frequent mutations, most of which are found in the spike gene. Understanding the underlying mutations is crucial to understanding the biological and epidemiological characteristics of the expanding family of SARS-CoV-2 variants. Although similar concerns have been expressed over the beta and gamma variants, preliminary data from the UK suggests that the alpha variety of SARS-CoV-2 is more contagious than earlier versions, and preliminary data indicates that there is a potential that infection will worsen the illness[15].

Additionally, some studies suggest that people who have already had the SARS-CoV-2 form may be less protected from getting the beta variant in the future[4]. This discovery raises the prospect that the variant may be more resistant to the currently available vaccines, as does another investigation showing an approximately 6-fold decrease in the neutralization of the Beta variant by sera from individuals who got a vaccine designed to protect against the wild-type virus. It should be stressed that this type of assessment, which uses neutralizing antibodies from individuals who have already received an immunization or been exposed to an infection, does not assess alternative potential forms of immunity, such as memory T- and B-cell activity.

The wild-type and the Alpha variant infections often result in the most severe and critical cases, even though most SARS-CoV-2 infections with the Beta, Gamma, and Omicron variants are mild to moderate in intensity. The infection with different SARS-CoV-2 variants was the subject of this inquiry. Due to the sample's collection date of March 2021 and the Alpha variation's dominance over the wild type, the Alpha variant had the highest infection percentage of 109 (46.6%). Then came the Beta or Gamma variations 47 (20.1%), the Omicron variant 46 (19.6%), the Delta or Delta plus variant 84 (26.33%), and ultimately the wild-type virus 32 (13.7%). During data collection, the Beta variant was the most prevalent illness worldwide (early March 2021).

In contrast, the Gamma variant was still undiagnosed, notwithstanding that the PCR variant kit cannot differentiate between beta and gamma variants in addition to Delta or Delta plus variants. Three people (50%) died as a result of the Beta variant, which can still result in severe and life-threatening illnesses. In severe and fatal cases, patients may also have additional comorbidities (diabetes, hypertension, cardiac problems, renal failure, etc.). The Gamma variant, which was discovered in late March 2021, spread more quickly than the Beta variant, attacked more people, and caused a high incidence of mild and moderate disease, which increased the ratio of mild illness and made it statistically relevant. Age and gender do not have significant relationships with the omicron variant, although the Ct value and infection severity do[16]. Patients under 45 were more likely than older patients to develop the Alpha variant compared to the wild-type (p 0.05). At the time of the sample collection for this study in March 2021, the Alpha variant started to have an advantage over the wild-type, infecting more people globally. The most severe SARS-CoV-2 infections, according to the current study's findings, however, appeared in the wild type and during the advent of the Alpha variety[17].

CONCLUSIONS

Older individuals are more likely to be exposed to more severe SARS-CoV-2 sickness. Males are more likely than females to contract COVID-19 and suffer severe consequences. The cycle threshold value was at its lowest during infection with the Alpha variant and wild-type virus. However, it peaked when the Beta, Gamma, Delta, Delta Plus, and Omicron varieties were infected. Patients with wild-type virus infections, the Alpha, Delta, and Delta plus variants, and those with Beta or Gamma infections and the Omicron variants, who had milder illnesses, faced more severe and potentially fatal diseases (lower Ct value). Late February of 2020 witnessed a preliminary finding of wild type in Iraq, which persisted throughout December of the same year. The inaugural Alpha variant emerged in December 2020, Beta or Gamma variances were initially recognized in December 2020, Delta or Delta plus variances began in April 2021, and finally, the Omicron variant debuted in March 2022. New sub-lineages continued to appear until this day.

Financial support and sponsorship: This research received no external funding

Conflict of interest: The authors declared no conflict of interest.

Sources of funding: This research received no specific grant from public, commercial, or not-for-profit funding agencies.

Author Contributions: Conceptualization, Hula Y. Fadhil.; Methodology, Jinan J. Ghazzi and Thaer A. Abdul Hussein; Software, Hula Y. Fadhil.; Writing original draft preparation, Hula Y. Fadhil, Jinan J. Ghazzi, Thaer A. Abdul Hussein and Zeyad K. Jabbar; supervision, Hula Y. Fadhil.

Institutional Review Board Statement: The study was conducted according to the Declaration of the College of Science Research Ethics Committee guidelines that approved the study protocol (Reference: CSEC10921/0042).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Received: May 22, 2023/ **Accepte**d: October 15, 2024 / **Published:**15 February 2024

Citation: Ghazzi J, Fadhil H, Abdul Hussein T, Jabbar Z. Detection of SARS-CoV-2 variants distributed from March 2020 to May 2022 and their effect on the infection severity in the Iraqi population. Bionatura Journal 2024; 1 (1) 41. http://dx.doi.org/10.21931/BJ/2024.01.01.41

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Peer review information. Bionatura thanks anonymous reviewer(s) for their contribution to the peer review of this work using https://reviewerlocator.webofscience.com/