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A Molecular Study of Covid-19 By Using ROF1b\N\E Gene in Wasit Province

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Abstract

This study aims to investigate the prevalence of COVID-19 in patients according to age, gender, and place, concentrating on the population aged 1 to 70 in Wasit province. A prospective controlled study included 600 patients (390 males and 210 females) who suffered from the following symptoms: headache, exhaustion, taste and appetite loss, fever, and shortness of breath from July to November 2023. Swab nasal samples were collected, and patient information was taken after obtaining consent from all patients; real-time reverse transcription PCR technology was then used to detect the coronavirus in patient samples. Results showed the total percentage of COVID-19 infection was 59/600(9.83%) in patients; according to age, the results indicated that the age group (16–25) had the highest proportion of COVID-19 infection (2.66%), while the age group (46–55) had the lowest percentage of infection (0.33%). In contrast, according to gender, the results showed the highest percentage of COVID-19 infection was (5.33%) for males, and the lowest percentage of

infection was (4.50%) for females. In conclusion, statistical analysis results indicated significant differences in infection between female and male patients.

Introduction

Single-stranded RNA viruses are called coronaviruses [1]. It is thought to have a zoonotic origin because isolates of the virus from bats and pangolins share genetic similarities with isolates from humans [2, 3]. In December 2019, China reported the spread of respiratory disease by the virus known as "Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2)". By January 2020, the outbreak there had soon turned into an epidemic, which expanded swiftly to different regions of the globe [4]. Over 757 million COVID-19 cases were confirmed by the end of February 2023, with over 6.85 million cases being fatal [5]. These cases were reported to the WHO. Furthermore, it was calculated that the pandemic was responsible for 14.83 to 14.9 million additional

fatalities in 2020 and 2021 [6,7, 37-39]. Iraq reported more than 24,000 deaths associated with COVID-19 and over 2.1 million confirmed cases as of September 29th, 2022 [8].

Humans may continue to exhibit symptoms or not at all after infection. Even though they are less contagious, asymptomatic people can still discharge a significant amount of the virus, making them efficient and undetectable carriers. COVID-19 incubates for three to fourteen days and is spread by aerosols, droplets, and sneezing [9]. Frequent signs and symptoms include bodily weakness, coughing, and fever. A few people experience diarrhoea, pharyngalgia, myodynia, runny nose, and congestion in their nasal passages. In extreme cases, dyspnea and/or hypoxia may emerge by the end of the first week of the illness [10]. Interestingly, patients with high numbers of viruses may experience a mild or non-existent fever during the illness. Some kids and infants did not exhibit the usual signs; instead, they showed up with digestive problems such as diarrhoea and vomiting, or they showed up depressed or breathless [11].

All age groups are typically at risk of acquiring COVID-19 infection. The virus is mainly spread by contact (touching the virus that is placed on an object's surface, and then passes through one's body through the nose, mouth, eyes, or other mucus membrane) or droplet (directly inhaling droplets from an infected person's sneezing, coughing, or talking) [12]. There has been no difference in virus load between patients with symptoms and those without, who can also spread the disease. Research has revealed that viral load in the cavities of the nose is more significant compared to the oral cavity, indicating that nasal collection is a more effective way to identify the virus [13].

The innate immune system can deteriorate with age, which could be a factor in developing severe coronavirus infection. In particular, older adults may have a lower level of innate immunity, which may lead to a higher load of coronavirus, which in turn can cause the adaptive immune system to become overactive and produce more cytokines [14, 15]. Rezaei et al. describe COVID-19 as "a single-strand positive-sense RNA virus composed of twenty-nine proteins and 29,903 nucleotides" [16]. The four structural proteins and sixteen non-structural proteins (NSPs) are encoded by SARS-CoV-2. The envelope and the capsid are made up of structural proteins, which include the nucleocapsid (N), envelope glycoprotein spike (S), envelope (E), and transmembrane (M) [17]. The primary requirement for viral replication is met by the non-structural proteins encoded by ORF1ab [18].

Rapid development has led to innovative nucleic acid detection techniques in molecular diagnostics. The SARS-CoV-2 virus, the infectious agent causing the current epidemic, can be identified using RNA to provide molecular identification of coronavirus. The original genome sequence of the coronavirus has been identified using metagenomic RNA sequencing, an objective, high-level throughput technique for sequencing multiple genomes [19, 20]. Quantitative reverse-transcription polymerase chain reaction (RT-PCR) techniques are the most common method used for COVID-19 molecular diagnosis globally. In various PCR assays, primer design has been used to target multiple conserved regions of the COVID-19 virus genome [21].

SARS-CoV-2 Structure

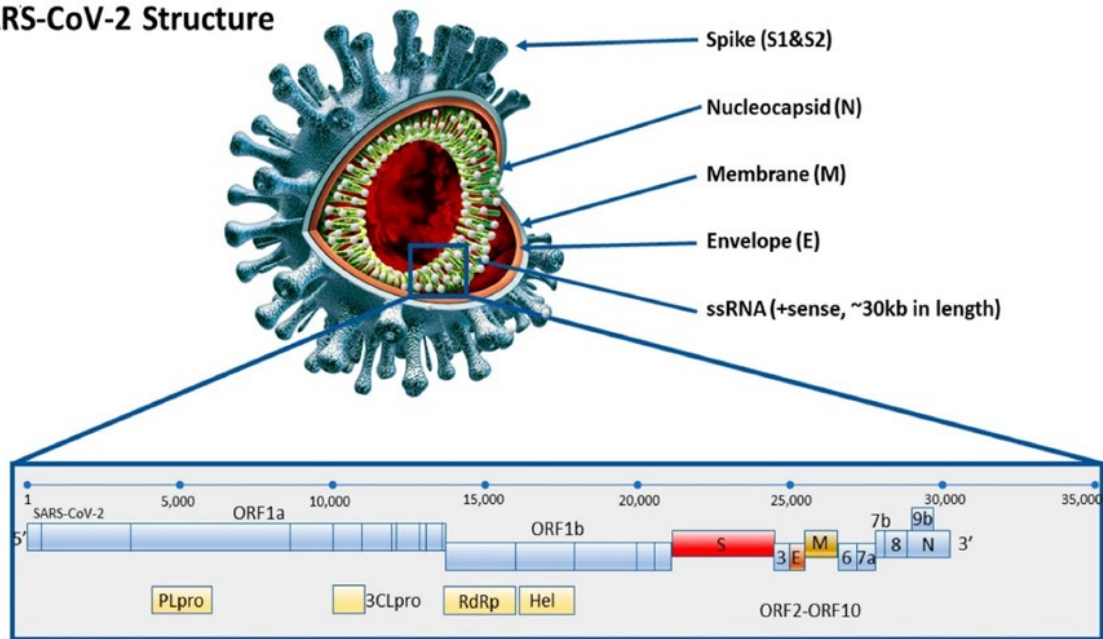


Figure (1-1): Coronavirus structure (Kubina & Dziedzic,2020)

Methods

Nasal swab samples were collected from 600 patients (390 males and 210 females) who suffered from fever, shortness of breath, fatigue, loss of taste & appetite, headache and sore throat during the period from July to November in 2023 at AL-Karama Educational Hospital and private medical clinics in Wasit province. The patient's information was collected after obtaining consent from all patients, which included the name, age, gender, and residential location of the patient. Swab nasal samples were taken and placed in a disposable viral collection tube with a viral preservation solution; the tail was thrown out with the lid tightened and stored at 2°C-8°C until used. Real-time reverse transcription PCR technology was then used to detect the coronavirus in patient samples using the SARS-CoV-2 Fluorescent PCR Kit (Maccura, China) according to a method found in this Kit.

Statistical Analysis

The data was statistically analyzed using the SPSS program (version 27) to obtain percentages. P values (0.05) in the data analysis were considered significant.

Results

Percentage of Covid-19 infection by using Real-Time PCR technique

During the study period, using the Real-Time PCR technique, 600 nasal swab samples were collected and taken to detect the ROF1b \ N \ E gene of the Coronavirus in patients. According to the current study's findings, the rate of COVID-19 infection was 59 positive samples with a total percentage of 9.83 %, shown in table (1-1), Real-Time amplification plots for ROF1b\N\E gene of the Coronavirus in patient samples, as it is noted that the gene amplification is evident in the positive samples above the threshold line(orange line) and the absence of in the negative samples below the Threshold line shown as Fig. (1-2).

Table (1-1): Percentage of Covid-19 infection by using Real-Time PCR technique

Total Number	No. of positive samples	Percentage
600	59	9.83%

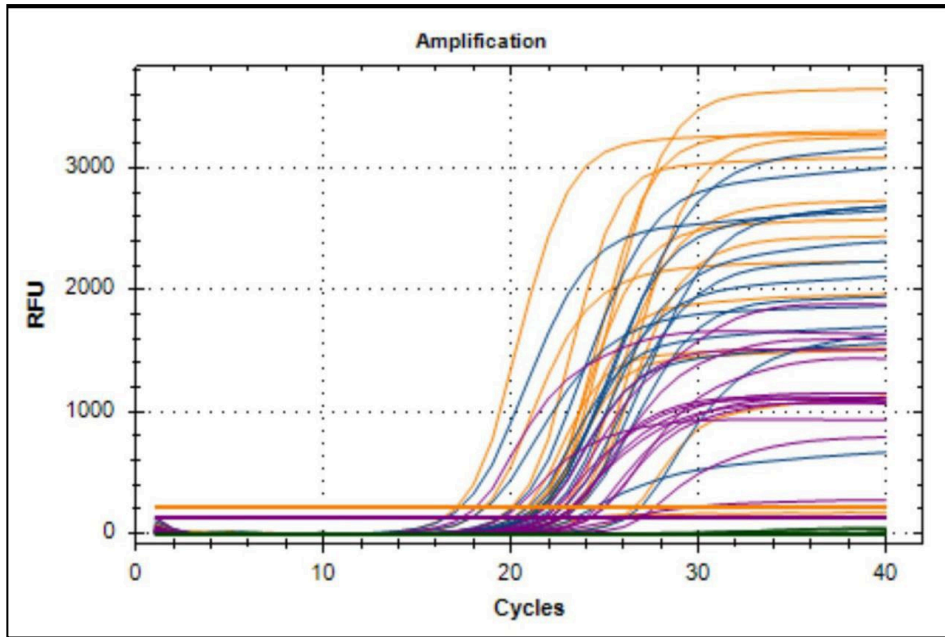


Figure (1-2): Plot showing the ROF1b/N/E gene amplification in the SARS-CoV-2 patient samples using Real-Time PCR

Prevalence of Covid-19 according to different ages

To ascertain the percentage of COVID-19 infection for different age groups using Real-Time PCR technology for 600 nasal swab samples from patients and divided into six categories for each category of 100 nasal swab samples, the results showed that the highest percentage was 2.66% in the age group (16-25), while lowest percentage of infection was 0.33% in the age group (46-55) which shown in Fig. (1-3).

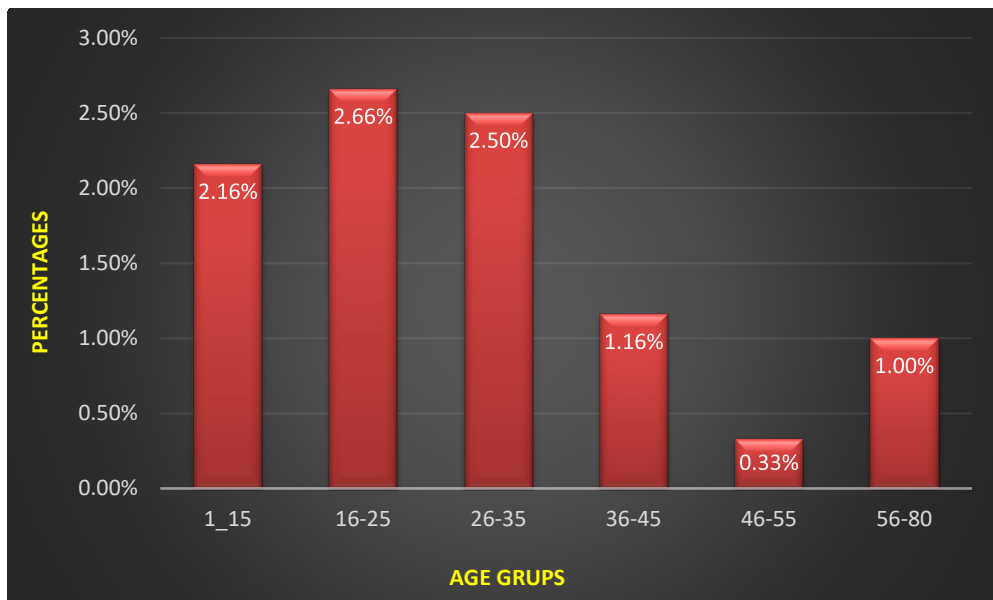


Figure (1-3): The relationship between the percentage of Coronavirus infection and the age of patients

Prevalence of COVID-19 According Gander

Out of a total of 600 nasal swab samples, including (390 males and 210 females) samples, the findings indicated that the highest percentage of infections was (5.33%) for males and the lowest rate of infection was (4.50%) for females, which is shown in Table (1-2).

Table (1-2): Prevalence of Covid-19 according to Gender

Gender	No. of positive samples	Percentage
Male	32	5.33 %
Female	27	4.50 %
Total	59	9.83%

Discussion

During this study period, a total of 600 nasal swab samples were collected and taken for detection of COVID-19 in patients by using Real-Time PCR technology. The results of the current study indicate that the rate of COVID-19 infection was 59 positive samples with a total percentage of 9.83%. There are many research results consistent with the results of the current study in terms of the presence of COVID-19 infections among many patients in Iraq. 148 patients at the Baghdad Medical City Complex's Ghazi AL-Hariri Hospital were examined using RT-qPCR; 19.6% showed positive results [22]. Furthermore, the current study's findings align with those of the study in Baghdad province, Iraq, which showed the patients' percentage of COVID-19 infection was 77.8% by using real-time PCR [8].

One of the main reasons for the COVID-19 predominance is its transmission mechanisms. Increased virus dissemination is brought on by several COVID-19 transmission mechanisms [12]. In essence, indirect and direct transmission are the two main categories [23]. The direct mechanism consists of three body fluids and secretions: faeces, saliva, urine, semen, and tears: (i) transmission via aerosols and (ii) mother-to-child. Two possible routes of indirect transmission are (i) things used on the sick individual and (ii) fomites or surfaces found in the immediate vicinity of an infected patient [23, 24]. Furthermore, many people have been reluctant to receive vaccinations, making it more challenging to develop herd immunity and stop the virus's transmission [25]. In outcome, there are several reasons for the high frequency of COVID-19, such as the absence of preventive measures, the virus's ease of transmission, people's restricted access to healthcare, the decline in vaccine protection, and the introduction of novel strains.

One factor that may predispose someone to COVID-19 is the patient's age. To determine the percentage of people with COVID-19 across different age groups, 600 nasal swab samples were divided into six categories of 100 samples each. The results showed the highest percentage was 2.66% in the age group 16–25, and the lowest percentage was 0.33% in the age group 46–55. Another study conducted in Iraq by Sarhan et al. revealed that COVID-19 can infect people of any age, with the maximum range being 19% (ages 30–39) [15]. According to a study conducted in Najaf City, Iraq, 33.1% of people aged 21 to 30 had COVID-19 infection [17]. This may be due to immunodeficiency, failure to take vaccines, and lack of adherence to safety precautions.

Another factor that may predispose someone to COVID-19 is gender. Out of 600 nasal swab samples, including 390 male and 210 female samples, the findings indicated that the most significant proportion of COVID-19 infections was 5.33% for males, and the lowest percentage of infection was 4.50% for females. According to Ad'hiah et al., the proportion of males among the 1,014 identified cases of COVID-19 in Iraq (Baghdad and Basrah) was more significant than that of females (60% vs. 40%), which aligns with these results [26]. Similarly, another study conducted in Iraq by Sarhan et al. discovered that 45% of confirmed cases were female and 54% were male [15]. In Najaf City, Iraq, 35.6% of cases were female and 64.4% male [17]. Additionally, 57.5% of 67,180 patients in a Brazilian study were male [16]. According to most epidemiologic studies and meta-analyses, males and females had similar COVID-19 rates [27–29]. The findings of the present research disagree with those of Hussein et al. in Duhok province, Iraq, which found the infection percentage in symptomatic patients was higher in females (54.68%) than males (45.35%) [30].

One possible explanation for the more significant COVID-19 morbidity in men could be that men are more prone to seek medical attention after the signs and symptoms first appear [31]. Men may also be at greater risk due to immunological variations, affecting their ability to fend off infections, including COVID-19 [32]. Females have greater innate and adaptive immune responses than males due to the X chromosome, which includes more copies of immunity-associated genes [33], potentially leading to more rapid clearance of SARS-CoV-2 in women reducing disease severity and mortality. Additionally, sex-dependent steroid hormone synthesis might contribute to gender-specific illness outcomes following viral infections [3, 34]. Direct evidence for sex variations in immune responses comes from recent findings that women exhibited more excellent IgG antibodies active against SARS-CoV-2 than men [35]. Previous clinical research has demonstrated that females produce fewer cytokines, are less likely to contract viral infections, and show stronger antibody responses; this may account for variations in COVID-19 susceptibility and outcomes between male and female patients [36].

Conclusions

Despite vaccines available for the coronavirus, treatment of complications resulting from infection with this virus, and safety precautions and preventive measures in Wasit province, the findings of this study that occurred in 2023 indicated a higher percentage of coronavirus infection in patients. The age group (16-25) recorded the highest infection rate from the other groups. Also, males had a higher infection rate than females. There are several reasons for the high frequency of COVID-19, such as the absence of preventive measures, people's restricted access to healthcare, the decline in vaccine protection, the introduction of novel strains and the virus's ease of transmission. The COVID-19 virus is very contagious, which might result in the infection spreading. Minimizing interpersonal communication, sanitizing commonplace items, practising good hygiene, regular hand washing, using face masks, and coughing and sneezing are all essential to stopping the virus's spread.

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