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Seroepidemiological Study of Novel Coronavirus Disease (COVID-19) in Tehran, Iran

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ABSTRACT

Backgrounds: A novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has now spread to all countries of the world, including Iran. Although anti-SARS-CoV-2 antibodies may be identified in patients using immunological methods with sufficient sensitivity and specificity, the conclusive diagnosis of the disease is made using the molecular RT-PCR method. A population-based seroepidemiological survey was conducted to quantify the proportion of the exposed population with SARS-CoV-2 antibodies and evaluate whether the antibodies are a marker of total or partial immunity compared to the population that remains susceptible to the virus.

Material & Methods: This cross-sectional study was conducted to investigate the seroprevalence of COVID-19 in Valiasr, Sajad, and Ghaem hospitals in Tehran, the capital of Iran, from April to the end of October 2020. Clotted and heparinized blood specimens (2mL) were collected from the patients. The serum and plasma were separated and stored at −80 °C until use. Anti-SARS-CoV-2 IgG and IgM antibodies were examined in the serum samples of 1375 in-patients admitted to the hospitals using ELISA kits. The obtained data were analyzed using SPSS software Ver.22.0 by employing statistical tests such as Chi-square and Fisher's exact tests. A p-value <.05 was considered as significant.

Findings: In total, 1375 participants were enrolled in this study, and SARS-CoV-2 antibodies were detected in 291 patients using IgM-IgG antibody assay. Among the seropositive patients studied, 187 were male (64.3%), and 104 were female (35.7%) (p<.05). The mean age of the patients was 49±8.4 years; the majority of whom (27%) were in the age group of 31-40 years. Also, the lowest frequency of infected cases was related to the age group of 1-10 years (p <.05). The seroprevalence of SARS-CoV-2 IgM or IgG antibodies was determined to be 21.2%. Diabetes mellitus was the most common underlying disease among SARS-CoV-2 patients [p=.05; Odd Ratio=1.61(0.90-2.91)].

Conclusion: The use of conventional serological assays, such as the enzyme-linked immunoassay (ELISA), for detecting specific IgM and IgG antibodies in SARS-CoV-2 patients has a high-throughput advantage while minimizing false-negative results obtained using the RT-PCR method. In this study, the seroprevalence of SARS-CoV-2 antibodies was determined to be 21%. Control of diabetes, among other influential factors, plays an important role in the management and control of COVID-19.

Keywords: SARS-CoV-2, ELISA, COVID-19, Antibodies, Seroprevalence, Iran, Tehran .

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Introduction

Since the emergence of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in Wuhan, China, on December 12, 2019, it has spread quickly across the world and developed into a pandemic [1]. Iran is one of the Middle East countries severely affected by the COVID-19 pandemic. The first cases of SARS-CoV-2 infection in Iran were reported in the city of Qom on February 19, 2020 [2]. COVID-19 disease manifestations are highly diverse, ranging from asymptomatic/ mild respiratory pneumonia to severe acute respiratory syndrome (SARS). Asymptomatic or subclinical cases of COVID-19 are one of the most important public health challenges because they spread the infection and remain undetected in the community. Therefore, vigilant control measures are necessary at every stage of the COVID-19 epidemic to prevent its spread and resurgence. Among other variables, age has been identified as a risk factor for a more severe course of the disease; younger people tend to have moderate or even asymptomatic presentations and therefore play an important role in spreading the infection [3]. Accordingly, one study revealed that 51% of the confirmed cases, including 10 ship crew and 308 passengers, were asymptomatic [4]. Although the definitive diagnosis of the disease requires real-time polymerase chain reaction (RT-PCR), immunological and serological tests could detect coronavirus antibodies with appropriate sensitivity and specificity in patients with a wide range of presentations (asymptomatic, mild, moderate, and severe) [5]. Combined detection of IgM and IgG is of great value to improve the sensitivity of early diagnosis of COVID-19 [6]. These immunoglobulins, which usually appear in the blood of patients 8 to 10 days after the initial symptoms, could be promptly detected by immunological and serological tests with acceptable sensitivity and specificity; thus, they could be considered as an important tool for early detection of asymptomatic carriers in an attempt to control the spread of the infection [7-9]. Tehran is the capital and the largest city in the country. It is the most densely populated city in the country and reportedly has the highest number of coronavirus cases. However, the incidence rate per 100,000 people is not as high as that of less densely populated provinces of Semnan, Qom, Markazi, Yazd, Mazandaran, Qazvin, Guilan, Alborz, and Isfahan. Thus, the use of public health awareness campaigns in large and densely populated cities, where most sensitive jobs are located, is of economic and social importance because the increase in the disease prevalence in these cities could impact on other cities in the country [2]. The proportion of people with SARS-CoV-2 antibodies could be quantified by a population-based seroepidemiological survey. The survey could provide information on the proportion of the exposed population, the population that remains susceptible to the virus, and whether the antibodies are a marker of total or partial immunity [10].

Objectives: This study aimed to determine the seroprevalence of SARS-CoV-2 antibodies and the risk factors associated with SARS-CoV-2 infection in Tehran to provide valid decision grounds for healthcare professionals to effectively prevent, control, and treat the infection.

Materials and Methods

Study design and samples: This cross-sectional study was conducted to investigate the seroprevalence of COVID-19 in Valiasr, Sajad, and Ghaem hospitals in Tehran, the

capital of Iran, from April to the end of October 2020. These hospitals were referral centers for patients inside and outside Tehran. After receiving informed written consent, 1375 patients with suspected COVID-19 were included in this study according to the Helsinki Declaration. Patients' blood samples were collected according to the infection control guidelines in the hospitals using adequate personal protective equipment. During sample collection, patient's demographic characteristics, including age, gender, occupation, and underlying diseases, as well as other essential information were collected through a questionnaire provided to the patients as well as from the patients' records, respectively.

ELISA assay: Both clotted and heparinized blood samples (2mL each) were collected from the patients. The serum and plasma were separated and stored at −80 °C until use. Anti-SARS-CoV-2 IgG and IgM antibodies were examined in the serum samples of 1375 participants using ELISA kits (IDEAL TASHKHIS Co, IR. Iran). To detect IgM antibody, 100 µL of diluted serum (1:50) was added into the wells of a 96-well microplate (coated with N protein) and incubated at 37 °C for 30 min. After washing, 100 µL of enzyme conjugate was added into the wells and then incubated at 37 °C for another 30 min. Following the second wash cycle, 100 µL of substrate was added into the wells and incubated at 25 °C for 15 min. Finally, a stop solution was added to the wells to terminate the reaction. The optical density (OD) of each well was determined by a microplate reader at 450 nm within 30 min. For detecting IgG antibody, the dilution factor was changed (1:100). According to the protocol, the sensitivity of the test was about 61% for IgM antibodies after 7-14 days and 82% for IgG antibodies after 11-15 days from the onset of infection. The performance of the kit was confirmed on 20 blood samples of PCR-negative volunteers (negative control) and 20 patients with definitive coronavirus (PCR positive) (positive control).

Statistical analyses: The obtained data were analyzed using SPSS software Ver.22.0 by employing statistical tests such as Chi-square and Fisher's exact tests. A *p*-value <.05 was considered as statistically significant.

Findings

In total, 1375 participants were enrolled in this study, and SARS-CoV-2 was detected in 291 patients using IgM-IgG antibody tests. Out of 1375 participants studied, 954 were male (69.4%), and 421 were female (30.6%) (p<.05). The mean age of the patients was 49±8.4 years; the majority of whom (27%) were in the age group of 31-40 years. Also, the lowest frequency of infected cases was related to the age group of 1-10 years (2%) (p <.05). Among all the participants, 291 patients (21.2%) were positive for either IgM or IgG antibodies, indicating a past or present infection (p <.05). Among 291 seropositive patients, 187 (64.3%) were male, and 104 (35.7%) were female (p<.05). The summary of serological findings is shown in Table 1.

A significant difference was observed between the age group over 60 years (as a risk factor) and the other age groups [p <.05; Odd Ratio = 2.72 (1.7-4.35)] (Table 2).

Table 1) Seroprevalence of new coronavirus patients by gender

Gender	IgM or IgG		Total N (%)
	Positive N (%)	Negative N (%)	
Male	187 (13.6)	767 (55.8)	954 (69.4)
Female	104 (7.6)	317 (23)	421 (30.6)
Total	291 (21.2)	1084 (78.8)	1375 (100)
***P*=.03, df=1**			

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Table 2) Seroprevalence of new coronavirus patients by age groups

	IgM or IgG		Total N (%)
	Positive N (%)	Negative N (%)	
0-10	6 (0.5)	16 (1.2)	22 (1.6)
11-20	15 (1.1)	76 (5.5)	91(6.6)
21-30	51 (3.7)	286 (20.8)	337 (24.5)
31-40	75 (5.5)	296 (21.5)	371 (27)
41-50	72 (5.2)	259 (18.8)	331 (24.1)
51-60	40 (2.9)	104 (7.6)	144 (10.5)
>60	32 (2.3)	47 (3.4)	79 (5.7)
Total	291 (21.2)	1084 (78.8)	1375 (100)
	***P*=.0001, df=6**		

Clinical and laboratory data of the patients were collected and analyzed. In this study, 57 patients (4%) had underlying diseases including diabetes, cardiovascular diseases, autoimmune disorders, respiratory diseases, etc. Diabetes mellitus was the most common underlying disease among SARS-CoV-2 patients [*p*=.05; Odd Ratio=1.61(0.90-2.91)]. Also, 28% of the participants were government employees, and the rest were self-employed or unemployed. The comparison between government employees and those with other occupations in terms of monthly incidence rate of COVID-19 new

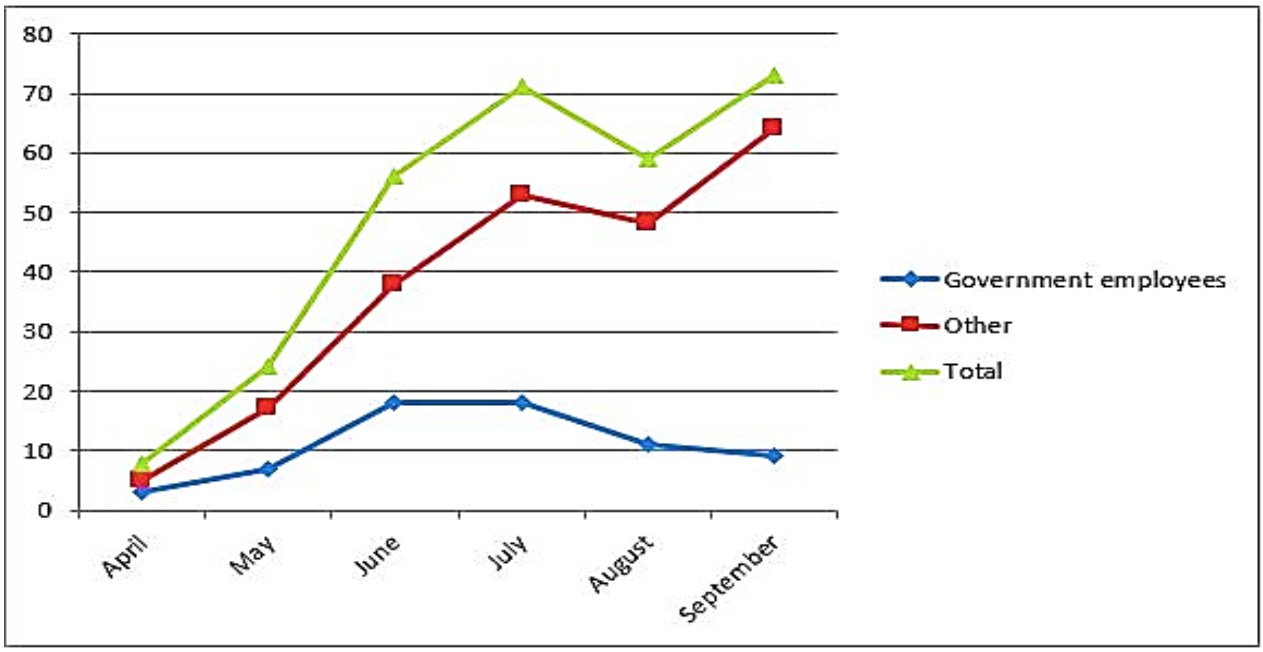

Figure 1) Comparison between government employees and those with other occupations in terms of monthly incidence rate of COVID-19 new cases

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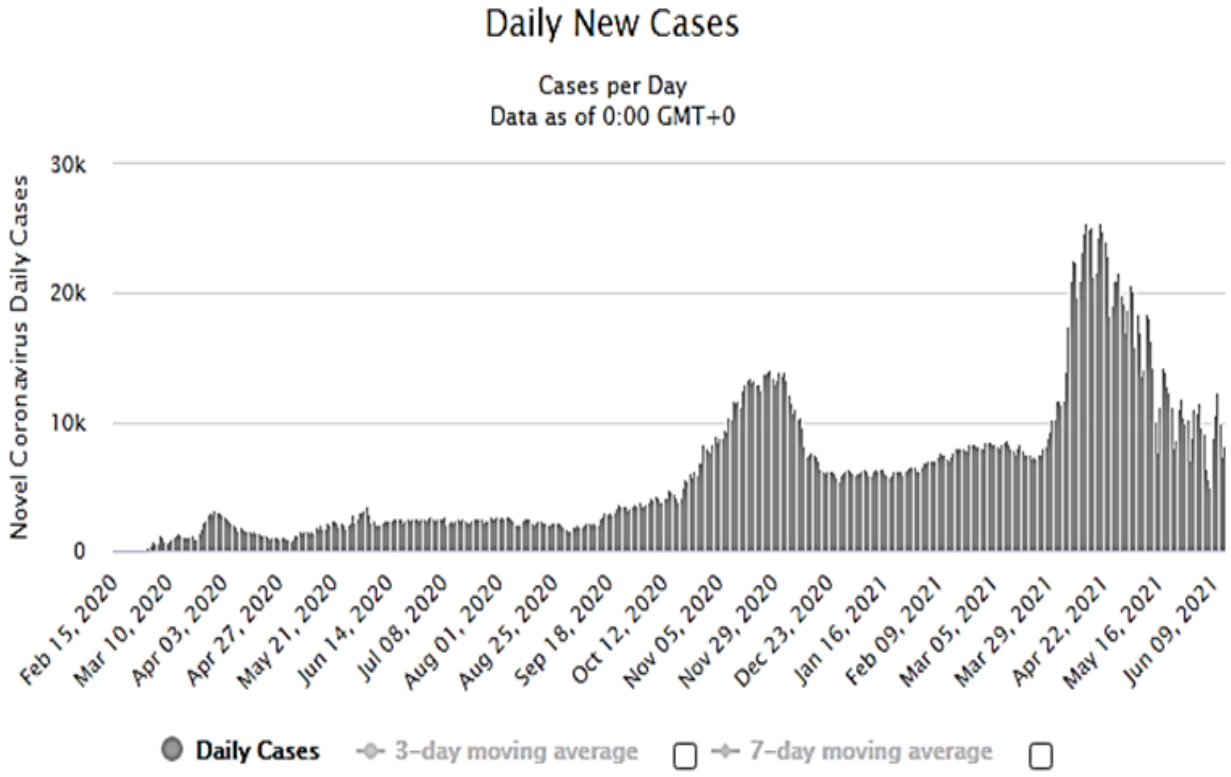
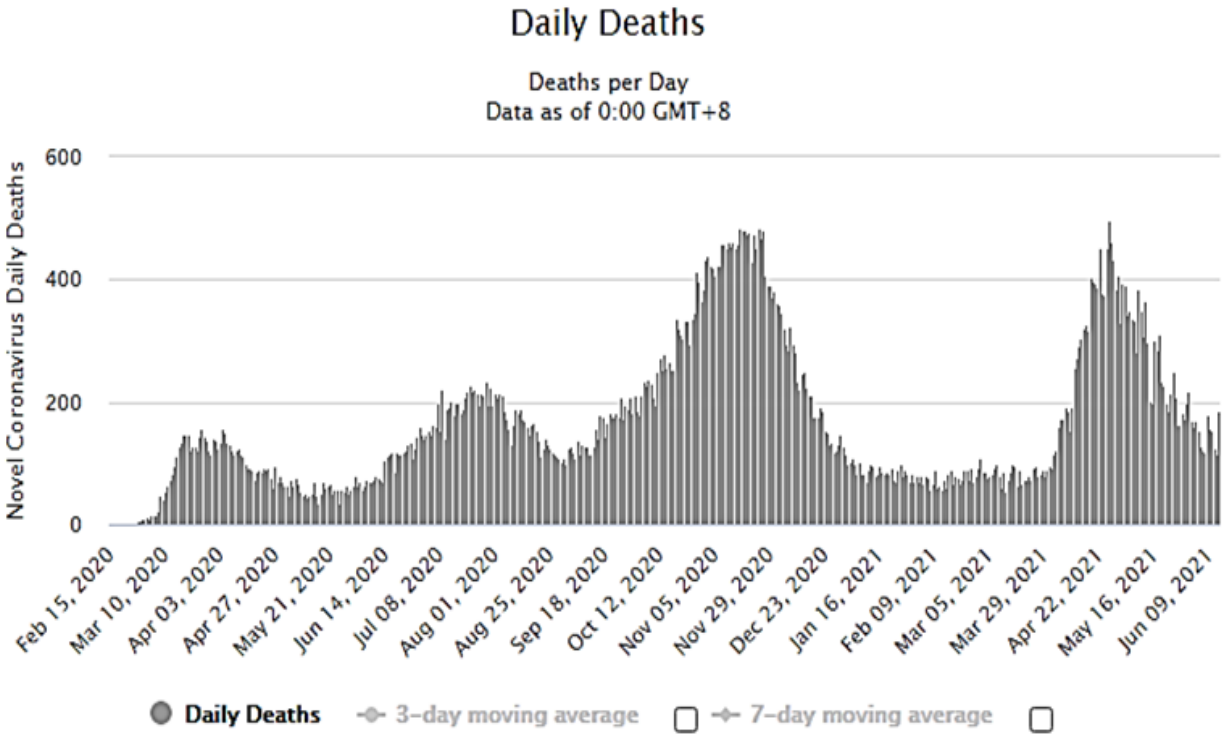
cases is shown in Figure 1.

Daily incidence rates of new cases and deaths due to COVID-19 in Iran from February 15, 2020 to Jun 9, 2021 are shown in Figure 2.

Among the seropositive patients, 66 (22.7%) were in the group of government employees, and 225 (77.3%) were among the other





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groups [p <.05; Odd Ratio = 0.79 (0.6-0.98)]

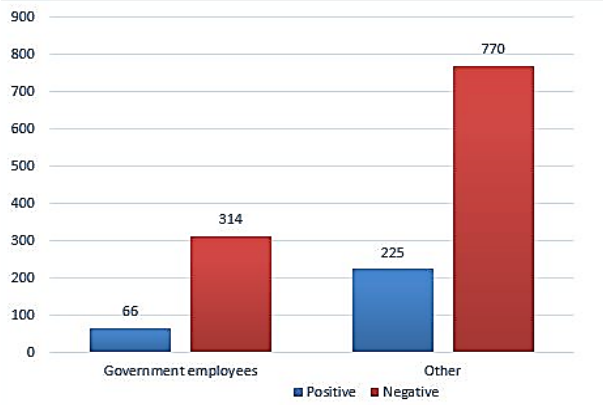

Figure 3) Seroprevalence of new coronavirus patients by the type of occupation

Discussion

COVID-19 emerged as a global threat, affecting 73,809,570 people and causing about 1,642,000 deaths worldwide as of December 16, 2020. According to WHO reports, the overall mortality rate due to COVID-19 was 2.3%. With more than 1,123,000 confirmed cases and more than 52,000 deaths as of December 16, Iran remains as one of severely affected countries in the Middle East. Individuals with undocumented illnesses, whether those who are asymptomatic and do not seek medical treatment or those who are infectious throughout the incubation phase, pose the greatest risk of transmission [3]. The proportion of asymptomatic patients reported in different studies varies greatly, ranging from 4 to 41% [11]. This finding reinforces the importance of aggressive measures to identify, treat, or isolate individuals with confirmed SARS-CoV-2 infection and limit their contacts with other people to halt the spread of the epidemic. Despite the definitive diagnosis of the disease by molecular RT-PCR, immunological and serological tests could detect coronavirus antibodies (immunoglobulins) in patients at different stages of the infection with acceptable sensitivity and specificity. A serological survey is a powerful tool to determine the spread of infectious diseases, particularly in the presence of asymptomatic cases or incomplete diagnosis of those with symptoms [10]. According to the current study results, the seroprevalence of SARS-CoV-2 antibodies in Tehran was 21.2%. This result is consistent with the finding of another study conducted in Guilan province [4], but much higher than the estimated seroprevalence in California, USA, which was between 2.49 and 4.16% [12]. Besides, by comparing the outcomes of serological methods and that of molecular tests (RT-PCR) performed in Iran, it was found that the results of molecular tests showed a lower prevalence rate of 12%; according to the report of the Ministry of Health of Iran, by the end of October, 4,599,554 tests were performed, of which 545,286 were positive [13]. The reasons for this discrepancy could be the ease of access to antibody testing, low cost, the desire of clients for cheaper and more accessible methods, and the presence of false-negative results in molecular testing [14-15]. Statistical analysis of monthly incidence rate of the disease showed that from the beginning of May to the end of October, the general frequency of infected cases was ascending (p <.05), but among government employees, after reaching a peak in July and August, it started to decrease while still rising among the unemployed or self-employed people (Figure 1). Mandatory adherence to health protocols among government employees could be one of the reasons for this downward trend after August [p<.05; Odd Ratio=0.79(0.6-0.98)]. These results indicate the commitment of government employees to successfully adhere to health protocols through a unified and coordinated management system. However, the lack of access of unemployed and self-employed people to the government's coordinated management system, impacted and increased

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the prevalence of the disease among them. In this study, the disease was more prevalent among men than women (p <.05) (Table 1), which may be due to their greater exposure to the disease. Due to the special culture of Iranians, men are more likely to work outside the home than women, they are more at risk of the disease, and even the mortality rate in men is higher than in women. In this study, the mean age of the patients was 49±8.4 years; the majority of whom (27%) were 31-40 years old. Also, the lowest frequency of infected cases was related to the age group of 1-10 years (2%) (p <.05). These results are in line with the results of a study conducted in Spain, and one of the possible causes of these results is that the age groups of 21 to 50 years are active age groups, and the higher incidence rate of the disease in these active age groups than in other age groups seems reasonable. However, the intragroup comparison of people over 60 years old showed that out of 79 patients, 32 (41%) had coronavirus infection compared to other groups at relatively high risk of infection [p <.05; Odd Ratio = 2.72 (1.7-4.35)] (Table 2). This highlights the need for more effective measures specifically tailored to the elderly. According to the statistical results, failure to observe health protocols increases the prevalence of the disease. General measures to prevent the spread of COVID-19 include effective quarantine and isolation of infected cases, close monitoring of primary contacts, travel restrictions, and personal safety measures. Besides, extension of health protocols to specific occupational groups and individuals with underlying conditions should be done by the authorities [16-19].

Conclusion

In this study, the seroprevalence of SARS-CoV-2 antibodies was determined to be 21% in Tehran. Also, the use of conventional serological assays, such as the enzyme-linked immunoassay (ELISA), for detecting specific IgM and IgG antibodies in SARS-CoV-2 patients was shown to have a high-throughput advantage while minimizing the false-negative results obtained using the RT-PCR method. It is hoped that the current findings could provide valid decision grounds for healthcare professionals to effectively prevent, control, and treat SARS-CoV-2 infections amid the current pandemic. Control of diabetes among other factors could play an important role in the management and control of COVID-19.

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Ethical permission: The work was approved by the Ethics Committee of Iran University of Medical Sciences (IUMS), Tehran, Iran (no. IR.IUMS.FMD.REC.1399.485). Informed consent was obtained from all participants included in the study.

Conflicts of interests: The authors declare no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Authors' contribution: Conceptualization: ZT, MZ; Data curation and formal analysis: SD, MM, ZB; Investigation: SA, MZ; Methodology and project administration: SA, MZ, SD, ZT; Supervision: OR; Validation: MZ, ZT; Writing of original draft: OR, MG; Writing, reviewing, and editing: OR, MG.

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Consent to participate: A written informed was obtained from all patients.

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