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The Impact of Covid-19 Outbreaks on Adherence to Hand Hygiene in A Medical Teaching Hospital: A Case of Iran

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Abstract

Background: The new coronavirus is the cause of the COVID-19 pandemic that began in Wuhan, China, in December 2019. Regarding its rapid transmission and high prevalence, hand hygiene and personal protective equipment in health centers are some of the recommended interventions to prevent the spread of this disease. The current study aimed to investigate the effect of COVID-19 disease-related hand hygiene protocols on the rate of adherence to hand hygiene.

Methods: This descriptive cross-sectional study was conducted in an educational hospital in southeast Iran. The rate of adherence to hand hygiene was evaluated among hospital departments and healthcare workers (HCWs) during and before the COVID-19 outbreak. Total hand hygiene was 8264 cases (736 HCWs) in the first six months of the new Iranian year during the COVID-19 outbreak (i.e., 2020-3-20 to 2020-9-21) and 4254 cases (372 HCW) at the same time in the past year (i.e., 2019-3-20 to 2019-9-21).

Results: There was a 22.72-point increase in the total rate of adherence to hand hygiene during the COVID-19 outbreak in different hospital departments (ranging from 9.23 to 58.87%). Furthermore, the real commitment rate to hand hygiene was greater than 70% in nurses, operating room technicians, laboratory staff, certified practical nurses, and physiotherapists during the COVID-19 outbreak.

Conclusions: Although the proper implementation and supervision of infection control protocols during the COVID-19 pandemic appears to increase the rate of adherence to hand hygiene, it is not good enough. Other strategies and policies should be considered to improve adherence to hand hygiene.

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INTRODUCTION

Unusual cases of pneumonia with unknown causes were reported in Wuhan, China, in December 2019 and quickly became a global pandemic. The agent of pneumonia is a new type of coronavirus called SARS-COV-2, and the World Health Organisation called it Coronavirus 2019 (COVID-19) (Sun, Lu, Xu, Sun, & Pan, 2020). COVID-19 is a unique and rare disease without specific treatment that has challenged and stopped the world as a hidden and invisible enemy (LQ Li et al., 2020). The most common symptoms are fever, cough, and nonspecific symptoms such as shortness of breath, headache, fatigue, and muscle cramps (Bernheim et al., 2020); acute respiratory syndrome, multiple organ failure, and even death can occur in severe cases (Chen et al., 2020). The reported mortality rate of COVID-19 disease is approximately 6.6% worldwide (Toyoshima, Nemoto, Matsumoto, Nakamura & Kiyotani, 2020). WHO estimates show that there are over 54.78 million reported cases of COVID-19 and 38.38.11 million recovered patients, with more than 1.324 million deaths as of November 15, 2020 (Organisation, 2020).

COVID-19 is highly contagious and is transmitted primarily through respiratory droplets and close contact, and all individuals are susceptible to the disease (Oi Zhou et al., 2020). To reduce the overall risk of transmission of acute respiratory infections, the World Health Organisation highly recommended infection control interventions, including avoiding close and direct contact with people with acute respiratory infections, respiratory hygiene, frequent hand washing, especially after direct contact with sick people or their surroundings, and avoiding unprotected contact with domestic and wild animals (Farnoosh, Alishiri, Hosseini Zijoud, Dorostkar & Jalali Farahani, 2020). In addition to providing personal protective equipment in health centers, personal hygiene, and frequent hand hygiene were trained and recommended due to the rapid transmission and high disease prevalence (Rafeemanesh, Rahimpour, & Memarzadeh, 2020).

The hands of healthcare providers are one of the primary sources of infection transmission that can act as a source of organisms. As a mediator of microorganisms, it has been recommended that all healthcare providers consider hand hygiene as the first step to preventing and controlling nosocomial infections (Hazavehei, Noryan, Rezapour Sahkolaee, & Moghimbayge, 2016). Hands must be thoroughly cleaned with soap and water or disinfectant solutions before and after any contact with the patient or equipment. Hand hygiene is the most effective way to prevent infection by killing colonized microorganisms on the hand skin (Nazari, Haji Ahmadi, Dadashzade & Asgari, 2011). Studies have shown a 55-point decrease in infection transmission by proper hand hygiene (Jefferson et al., 2009; Najafi Ghezeljeh, Abbasnejad, Rafii & Haghani, 2015) and improper hand hygiene will lead to the spread of nosocomial infections and therefore increase costs and mortality rate (Holmen et al., 2016).

The growing rate of nosocomial transmission of COVID-19 disease increases the global demand for training and implementing more effective infection prevention and control strategies, such as using personal protective equipment and hand hygiene in all health facilities (Cook, 2020). Healthcare workers are at the forefront of the COVID-19 outbreak, and their permanent exposure to infected patients and contaminated surfaces can put them at risk of infection and transmission. The WHO has recently developed five moments for hand hygiene, including before patient contact, before aseptic task, after risk of exposure to body fluids, after patient contact, and after contact with patient surroundings (Lotfinejad, Peters & Pittet, 2020).

Although hand hygiene techniques are simple, adherence to hand hygiene is very challenging, and numerous studies indicate low acceptance and poor performance of health personnel in this regard (Albughbish, Neisi, & Borvayeh, 2016; Allegranzi & Pittet, 2009). WHO surveys showed that physicians and nurses adhered to hand hygiene in less than half of cases before COVID-19 disease, and hand hygiene was adhered to in less than 10% of patients in hospitals with a large workload (Hazavehei et al., 2016). A systematic review study in different countries in 2010 reported that 48% of nurses and 32% of physicians adhered to hand hygiene (40%) (Erasmus et al., 2010). A study in the US (2020) showed a 56-point increase in hygiene adherence during the Covid-19 epidemic (Moore, Robbins, Quinn, & Arbogast, 2021). Concerning the importance of hand hygiene, the COVID-19 pandemic, and the lack of study on the effect of COVID-19 on hand hygiene, this study was conducted to investigate the impact of the COVID-19 disease on adherence to hand hygiene in Iran.

Materials and Methods

Study Design and setting

The descriptive cross-sectional study aimed to investigate the effect of COVID-19 on adherence to hand hygiene at Shahid Bahonar Hospital in southeastern Iran from 2019 to 2020. Research settings include intensive care units (one CCU and four ICUs), medical-surgical units (two orthopedic units, two neurosurgery units, two oncology units, one urology unit, two surgical units (general and maxillofacial), one medical unit), emergency unit, operating room (four teams), laboratory, radiology, and physiotherapy of Shahid Bahonar hospital in Kerman. Bahonar Hospital is the first and largest trauma center in southeast Iran. It has 350 beds with the most advanced devices, imaging services, PACS system, telemedicine, flow cytometry, the most advanced neuro monitoring device, various cerebrovascular surgeries, brain aneurysms, etc.

Sample Size and Sample Sampling

The study population consisted of all hospital personnel who cared for a patient or, in some way, provided patient care. The sample size was 372 individuals in the first six months of 2019, including 1 nurse staff (144 nurses and 36 LPNs) and 2 nurses and cleaning staff (60 individuals). 3physician staff (4 cardiologists, 12 anesthesiologists, and 76 residents) and 4- other medical and paramedical staff (12 physiotherapists, four radiologists, 12 operating room technicians, and 12 laboratory technicians) and 736 individuals in the first six months of 2020, including 1nurse staff (304 nurses and 64 LPN), 2- nursing assistant & cleaning staff (112), 3- physician staff (8 cardiologists, 24 anesthesiologists and 144 residents) and 4- other medical and paramedical staff (24 physiotherapists, eight radiologists, 24 operating room technicians, and 24 laboratory technicians). All hospital wards' hand hygiene data in the first six months of 2020 (from April to September) were compared with the same time in the previous year (from April to September 2019) during the COVID-19 outbreak.

Measurement

In this study, a standard hand hygiene design based on the World Health Organisation was used to collect information (Alshammari, Reynolds, Verhougstraete, & O'Rourke, 2018; Pittet, Allegranzi, & Boyce, 2009). This checklist is used as an observational tool that examines the performance of four professional groups, including 1nurses, 2- nursing assistants, 3- physicians, and 4- other medical and paramedical personnel while providing health services. The checklist has designed four types of hand hygiene actions: hand rubbing, handwashing, missed hand rubbing, and gloves. Each observational session lasted 20±10 minutes. In the case of open and direct observation of adherence to hand hygiene, the observer should introduce himself to the professional staff and explain the purpose of the study. If repetition of hand hygiene moments is required, an observer can simultaneously observe a maximum of three medical staff members from one of the four professional groups.

A specific professional group scores hand hygiene in each checklist column, and we can record the performance of several staff in a similar professional group in one column. Similar teams in each of the professional groups include one nurse and RN, two nursing assistants3- internal medicine doctor, surgeon, anesthesiologist, emergency medicine specialist, pediatrician, cardiologist, consultant, and medical student in the professional medical group, and four physiotherapists, technician (radiology, operating room, anesthesiology, and laboratory) and a student in the paramedical group.

Each hand hygiene moment refers to a row in a column; each moment is defined with at least one indication (before patient contact, before aseptic task, after risk of exposure to body fluid, after patient contact, and after contact with patient surroundings). The wearing of gloves is marked if personnel use gloves and refuse to adhere to hand hygiene. For the initial calculation of the rate of commitment to hand hygiene in each professional group, the number of attempts to use hand hygiene is determined based on the five moments in each column of the professional group and multiplied by 100. The Ministry of Health, Treatment, and Medical Sciences of Iran has confirmed the reliability and validity of this instrument (Kavakebi et al., 2016; Mostafazadeh-Bora, Bahrami & Hosseini, 2018).

Strategies for adherence to hand hygiene and infection control in the research setting

Infection control programs such as triage strategy, congestion reduction, rearrangement of wards and environmental health, preparation and distribution of appropriate personal protective equipment, training workshops, educational posters, observation, and positive feedback for adherence to hand hygiene were implemented in different wards of the hospital spread to prevent the disease and increase hand hygiene.

Data Collection and Data Analysis

After obtaining the ethics code from the Ethics Committee of Kerman University of Medical Sciences and obtaining permission from the hospital manager, the researcher referred to the relevant departments, explained the objectives, importance, and process to the participants, and obtained their informed consent. In this study, the observer (Nosocomial Infection Control Supervisor) referred to the relevant wards and directly observed the five moments of hand hygiene in the morning shift. Each observational session lasted 20±10 minutes, and the observer simultaneously examined two members of the healthcare personnel. Hand hygiene moments they had ranged from 149 to 216 cases in each ward in 2019 and from 144 to 211 cases in 2020. They were included in the relevant checklist, and then the rate of adherence to hand hygiene and the number of cases of adherence to hand hygiene were calculated based on the five moments of hand hygiene. Excel (Version 2013) was used for data analysis. Frequency and percentage were used to describe the adherence rates to hand hygiene during and before the COVID-19 outbreak. The percentage change [(new value - old value)/old value)) * 100] was used to describe hand hygiene adherence rate changes between healthcare workers (HCWs in different hospital units) during and before the COVID-19 outbreak.

Ethical Considerations

This study received ethical approval and the research process protocol from the Nursing Research Centre, Department of Critical Care Nursing, Razi Faculty of Nursing and Midwifery (IR.KMU.REC.1398.581).

Results

The total performance of hand hygiene was 8 246 cases in the first six months of the new Iranian year during the COVID-19 outbreak (that is, 2020-3-20 to 2020-9-21), which was nearly two times higher than at the same time in

the past year (that is, 2019-3-20 to 2019-9-21). The total rate of adherence to hand hygiene was 72.69% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 22.72% higher than at the same time in the last year. The rate of adherence to hand hygiene ranged between 63.20% (radiology department) and 79.90% (Intensive care units) in the first six months of the new Iranian year during the COVID-19 outbreak, while it ranged between 39.78% (radiology department) and 71.29% (medicine unit) at the same time in the last year (Table 1).

The total rate of adherence to hand hygiene among nurses was 82.17% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 17.81% higher than at the same time in the last year. The total rate of adherence to hand hygiene among licensed practical nurses was 74.17% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 16.10% higher than that at the same time in the previous year. The total rate of adherence to hand hygiene among nursing assistants was 61.36% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 36.51% higher than at the same time in the past year. The total rate of adherence to hand hygiene among physicians and residents was 63.90% in the first six months of the new Iranian year during the COVID-19 outbreak, which was

35.18% higher than at the same time in the past year. The total rate of hand hygiene among operating room technicians was 77.88% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 13.06% higher than at the same time in the past year. The total rate of adherence to hand hygiene among anesthesiologists was 69.73% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 30.09% higher than the same time in the past year. The total rate of adherence to hand hygiene among radiology personnel was 63.20% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 58.87% higher than that at the same time in the past year. The total rate of adherence to hand hygiene among physiotherapists was 70.25% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 41.46% higher than at the same time in the past year. The total rate of adherence to hand hygiene among laboratory staff was 75.97% in the first six months of the new Iranian year during the COVID-19 outbreak, which was 28.76% higher than at the same time in the past year. The total rate of adherence to hand hygiene was more than 70% in nurses, operating room technicians, laboratory staff, certified practical nurses, and physiotherapists during the COVID-19 outbreak (Table 2)

Table 1: Adherence to hand hygiene before and during the COVID-19 outbreak in different hospital units.

Time	The first six months of the new		The first six months of a new year		Percentage
	year (Before the	e COVID-19)	(During the COVID-19)		changes
Variable	Number of	Percentage of	Number of	Rate of	
	observations (need	Adherence to	observations	Adherence to	
	for hand hygiene)	Hand Hygiene	(need for hand	Hand Hygiene	
			hygiene)		
Intensive care units	784	59.78	1611	79.90	33.66
Critical care unit	189	69.31	390	75.71	9.23
Emergency unit	182	57.69	385	69.60	20.64
Neurosurgery units	359	70.71	397	79.34	12.20
Orthopedic units	408	59.77	815	74.14	24.04
Oncology units	309	60.52	631	68.44	13.09
Surgical units	320	59.78	662	70.30	17.60
Medical unit	202	71.29	399	77.93	9.31
Orology unit	205	54.15	428	67.06	23.84
Operating rooms	768	59.77	1490	73.13	22.35
Radiology	181	39.78	340	63.20	58.87
department					
Physiotherapy	147	49.66	316	70.25	41.46
department					
Laboratory	200	59.0	382	75.97	28.76
department					
Total	4254	59.23	8246	72.69	22.72

Table 2: healthcare workers' adherence to hand hygiene before and during the COVID-19 outbreak.

Time	The first six months of a new year (Before COVID-19)		The first six months of a new year (During COVID-19)		Percentage changes
Variable	Number of	Percentage of	Number of	Rate of	
	observations	Adherence to	observations	Adherence to	
((need for hand	Hand Hygiene	(need for hand	Hand Hygiene	
	hygiene)		hygiene)		
Nurses	1709	69.75	3234	82.17	17.81
Licensed practical nurses	473	64.34	816	74.7	16.10
nursing assistant & cleaning	staff 515	44.95	1059	61.36	36.51
Physicians and residents	704	47.27	1468	63.90	35.18
Operating room technicians	153	68.9	300	77.88	13.03
Anesthesiologist technicians	172	53.60	331	69.73	30.09
Radiology staff	181	39.78	340	63.20	58.87
Physiotherapists	147	49.66	316	70.25	41.46
Laboratory staff	200	59.0	382	75.97	28.76

Discussion

In the present study, the rate of adherence to hand hygiene was examined during the outbreak of the Covid-19 epidemic and compared to the same time last year. The study results showed that the number of hand hygiene observations doubled in the first 6 months of the epidemic and that there was a 13.06-point increase in the total rate of hand hygiene in the hospital compared to the previous year. Adherence to hand hygiene and other infection control techniques appears to increase during infectious disease crises because hand hygiene is a priority for infection control, and healthcare workers are concerned about their health and infection transmission to others (Roshan, Feroz, Rafique & Virani, 2020). A 2009 study in China during the SARS epidemic showed that adherence to hand hygiene while caring for patients significantly reduced the transmission of infection between healthcare workers and patients (Morrison & Yardley, 2009). The meta-analysis results showed a 24-point reduction in COVID-19 transmission after adherence to hand hygiene (Kantor, 2020). A 2020 study during the COVID-19 epidemic in Karachi, Pakistan, found an increase in hand hygiene, as well as a decrease in nosocomial infections because staff were more aware and concerned about disease transmission to homes and people at risk of coronavirus, such as newborns, children, and elderly family members (Roshan et al., 2020). Improvement in knowledge and continuous education of healthcare personnel, along with effective infection control techniques, is essential in reducing the number of infections (Sarani, Balouchi, Masinaeinezhad & Ebrahimitabs, 2016). A study in Kuwait (2013) carried out an educational program on proper indications and techniques, used posters in this field, and showed a 61.4point increase in the rate of adherence to hand hygiene. Nurses adhered to hand hygiene more than other staff (82.5% after the educational program versus 49.9% before). The results showed that although cross-infection in the ICU was a complex process, strict adherence to hand hygiene recommendations could affect the number of infections (Salama, Jamal, Al Mousa, Al-AbdulGhani & Rotimi, 2013). A study in Indonesia (2017) implemented roleplaying training programs. It showed increased awareness of hand hygiene, but there was not enough, and more training programs focused on hand hygiene barriers were necessary (Santosaningsih et al., 2017). A study in Finland (2013-2018) showed that direct observation of hand hygiene and immediate feedback to health workers not only increased the rate of adherence to hand hygiene adherence from 76.4% to 88.5% but also decreased the rate of nosocomial infections (Ojanperä, Kanste & Syrjala, 2020). Direct observation helps determine the strengths and weaknesses of adherence to hand hygiene in healthcare workers and the number of hand hygiene moments, and it gives appropriate feedback to healthcare workers (Pittet et al., 2009).

According to the results of the present study, the highest rate of adherence to hand hygiene was related to the COVID-19 medical ward before the outbreak (71.29%). A study in Saudi Arabia (2011) showed that after increasing staff awareness, which is one of the standards for improving hand hygiene, the highest rate of hand hygiene was observed in patient care technicians in medical wards (100%) and very poor adherence was reported in other neighborhoods of the hospital. Therefore, infection control programs require much more attention in the future (Al-Mendalawi & Bukhari, 2011). A study in Iran (2008) found that 34.4% of medical staff and 15.7% in ICUs adhered to hand hygiene. This study showed that ICU staff underestimated hand hygiene due to emergency work conditions and high reactions while caring for patients; even hand hygiene was not adhered to when there was

insufficient time. Therefore, specific hand hygiene training follow-up for staff-related infections recommended (Samadipour, Daneshmandi & Salari, 2008). Patients admitted to intensive care units are more at risk of disease due to risk factors such as multiple injuries, depressed levels of consciousness, and weak defense mechanisms. Intensive care units around the world always have a low level of adherence to hand hygiene (hardly more than 40%) due to the higher priority of work conditions over hand hygiene, short time, limited facilities, sensitivity or intolerance to hand hygiene solutions and mismanagement (Tajabadi, Parsaeimehr & Kashani, 2018). However, the ICUs in our study adhered to hand hygiene during the outbreak of COVID-19 (79.90%), which was consistent with the Wuhan survey of China. In this study, the mean total adherence to hand hygiene was generally high (79.44%), and the highest rate of adherence to hand hygiene was observed in the intensive care unit (85%) due to the increased risk of the disease and its contagion, as well as direct education and observation of adherence to hand hygiene (Zhou, Lai, Zhang, & Tan, 2020). A 2018 study in the neurosurgical ICU used an infection control program, including hand hygiene, contact precautions, isolation of infected patients, and disinfection of the surroundings. It showed decreased nosocomial infections and antibiotic resistance and increased patient survival (Ershova et al., 2018).

In the present study, the lowest rate of adherence to hand hygiene was related to the radiology department both during the outbreak of COVID-19 (63.20) and at the same time in the last year (39.78), which was consistent with the results of the above studies. Researchers have found that standard precautions and hand hygiene are mandatory even in radiology, a place traditionally considered low risk in the transmission of pathogens, and radiology units should promote adherence to hand hygiene through appropriate training programs and the provision of hand hygiene products (Bibbolino, Pittalis, Schinina, Rizzi & Puro, 2009; Tsukamoto et al., 2006).

In this study, the nurse, operating room technician, LPN, laboratory staff, and physiotherapist adhered primarily to hand hygiene (more than 70%) during the COVID-19 outbreak. Researchers in systematic review studies (2011-2015) found that non-adherence to hand hygiene guidelines was a global problem, and monitoring and care methods should be standardized. Furthermore, most studies showed nurses adhered to hand hygiene more than physicians. They found that nurses often followed general guidelines for hand hygiene, while physicians were more likely to follow the correct hand hygiene technique (Erasmus et al., 2010; Gibson & Markovic, 2015). Since nurses directly care for patients, they play an essential role in preventing nosocomial infections, especially during outbreaks of infectious diseases, and their frequent contact with pathogens, long working hours, stressful environment, and fatigue predispose them to acquire or transmit infections such as Covid-19 (Heymann & Shindo, 2020). A study in China (2020) demonstrated that nurses and those with low burnout and high job satisfaction adhered to hygiene compared to physicians during the COVID-19 outbreak. Therefore, the study focused on interventions for higher job satisfaction and lower burnout (Zhou, Lai, Wan, Zhang & Tan, 2020).

Because nurses spend much more time with patients than other healthcare workers, a solid therapeutic relationship is established between the nurse and the patient, and patient safety leads to greater adherence to hand hygiene (Sands & Aunger, 2020). A study in Brazil (2017) revealed that the lowest rate of adherence to hand hygiene was related to medical residents (41.4%). The highest rate of adherence to hand hygiene was related to nurses and physiotherapists (6.66%) because the number of physiotherapists working in the study ward was small. Nurses were always in direct contact with patients (Zottele, Magnago, Dullius, Kolankiewicz & Ongaro, 2017). Shaykh al-Islami et al. (2014) found that nurses and cleaning staff (14.31%) and physicians (13%) adhered mainly to hand hygiene. They suggested special educational measures to accept physicians as models for other healthcare workers. However, unlike our research, the mean adherence to hand hygiene was zero in the physiotherapy and laboratory group, which is a warning sign and requires further studies on hand hygiene in this group due to its direct contact with patients and their essential roles in the spread of nosocomial infections (Ziasheikholeslami, Razavian, Vafaei & Esmkhani, 2016). Unlike our study, a study in China (2012) showed a low rate of adherence to hand hygiene in laboratory staff compared to physicians and nurses. Therefore, hand hygiene training should be increased, hand hygiene facilities should be improved, and management systems should improve adherence to hand hygiene (X. Li, 2012). A 2017 study in Karachi, Pakistan, demonstrated the low rate of adherence to hand hygiene among surgeons and operating room technicians, which increased after video surveillance and direct feedback (Khan & Nausheen, 2017). Our study's increased adherence to hand hygiene may be due to continuous education, increased awareness, and the use of appropriate and effective infection control techniques due to rapid transmission and fear of the disease. One of the limitations of the present study was its implementation in a trauma hospital, which is not a reference hospital for patients with COVID-19. Therefore, hospitals that admit patients with COVID-19 may have a different rate of adherence to hand hygiene.

Furthermore, the study was conducted in the early months of the prevalence of COVID-19 disease, when the adherence to health protocols and hand hygiene could be different. Another limitation of the study was that observations were made in the morning shift. Additional conditions of night and evening shifts, lack of continuous

supervision, and visiting hours may affect hand hygiene adherence in evening and night shifts. In other words, the rate of adherence to hand hygiene in different shifts may be different, which should be considered in future studies.

Conclusions

The results of this study indicate that although infection control and hand hygiene strategies during the coronavirus epidemic led to greater adherence to hand hygiene in various departments and healthcare personnel, they are still not enough. Therefore, it is suggested that further studies be conducted to review and provide appropriate assessment and monitoring strategies and increase staff motivation to follow proper hand hygiene protocols that help break the chain of infection. Furthermore, the current situation should be examined in terms of the knowledge and performance of health workers, more training courses should be considered, and the importance of hand washing should be fully explained to them.

DECLARATION SECTION

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Ethical Considerations

This study received ethical approval and the research process protocol from the Nursing Research Centre, Department of Critical Care Nursing, Razi Faculty of Nursing and Midwifery (IR.KMU.REC.1398.581).

Conflict of interest

None to be declared.

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Data availability

Data are available by contacting the corresponding author by email.

Authors contribution

All authors have read and approved the manuscript.

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