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The Effect of Prone Position on the Development of Ventilator-Associated Pneumonia in COVID-19 Patients in Intensive Care Unit: A Cross-Sectional and Case-Control Study

Yoğun Bakım Ünitesindeki COVID-19 Hastalarında Yüzüstü Pozisyonun Ventilatörle İlişkili Pnömoni Gelişimine Etkisi: Kesitsel ve Vaka Kontrol Çalışma

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ABSTRACT Objective: To determine the effect of position on the development of ventilator-associated pneumonia (VAP) in coronavirus disease-2019 (COVID-19) patients in intensive care unit. **Material and Methods:** This cross-sectional, retrospective, and case-control study included 138 COVID-19 patients intubated in the medical-surgical intensive care unit of Koç University Hospital in March 2020-2021. Multiple logistic regression analyses were conducted to determine the factors affecting VAP development. **Results:** In the regression analysis, the affecting variables the VAP incidence among all COVID-19 patients were prone position, hypertension (HT) disease, and other comorbid diseases ($p<0.05$). Further logistic regression analysis among prone-positioned COVID-19 patients showed that the duration of intubation odds ratio (OR) 1.14, 95% Confidence Interval (CI) 1.04-1.25), smoking (OR 8.14, 95% CI 1.29-51.16), and having other comorbid diseases (OR 0.15, 95% CI 0.03-0.70) increased the risk of VAP development. It was also found that the incidence of VAP decreased as the prone time increased (OR) 0.68, 95% CI 0.52-0.89) ($p<0.05$). **Conclusion:** The risk for VAP development increases in prone positioned patients with HT and other comorbid diseases and VAP develops often due to *Acinetobacter baumannii*. In prone position, the risk of VAP increased in patients with prolonged intubation time, smokers, and other comorbid diseases, and decreased as the prone time was prolonged. It is important to maintain care approaches included in VAP bundles, such as oral care of patients in prone positions to reduce the incidence of VAP secondary to COVID-19 infection, especially during the first days of intubation.

Keywords: Prone; supine; ventilator-associated pneumonia; acute respiratory distress syndrome; COVID-19

ÖZET Amaç: Bu çalışmanın amacı, yoğun bakım ünitesinde yatan koronavirüs hastalığı-2019 [coronavirus disease-2019 (COVID-19)] hastalarında pozisyonun ventilatör ilişkili pnömoni (VİP) gelişimine etkisini belirlemektir. **Gereç ve Yöntemler:** Bu kesitsel, retrospektif ve vaka kontrol çalışmasına Mart 2020-2021'de Koç Üniversitesi Hastanesi yoğun bakım ünitesinde entübe edilen 138 COVID-19 hastasını dâhil edildi. VİP gelişimini etkileyen faktörlerin belirlenmesi amacıyla çoklu lojistik regresyon analizleri yapıldı. **Bulgular:** Regresyon analizinde tüm COVID-19 hastalarında VİP görülme sıklığını etkileyen değişkenlerin pron pozisyonu, hipertansiyon (HT) varlığı ve komorbid hastalıklar olduğu bulundu ($p<0,05$). Ayrıca, pron pozisyondaki hastalar arasında ileri lojistik regresyon analizi, entübasyon süresinin odds oranı (OR 1,14, %95 CI 1,04-1,25), sigara içmenin (OR 8,14, %95 CI 1,29-51,16) ve diğer eşlik eden hastalıklara sahip olmanın (OR 0,15, %95 CI 0,03-0,70) VİP gelişme riskini artırdığı bulundu. Son olarak, pron süresi arttıkça VİP sıklığının azaldığı belirlendi (OR 0,68, %95 CI 0,52-0,89) ($p<0,05$). **Sonuç:** Yüzüstü pozisyonda HT ve eşlik eden diğer hastalıkları olan hastalarda VİP gelişme riski artmakta ve VİP sıklıkla *Acinetobacter baumannii*'ye bağlı olarak gelişmektedir. Yüzüstü pozisyonda, entübasyon süresi uzamış, sigara içen ve komorbid hastalıkları olan hastalarda VİP riski artmakta, pron süresi uzadıkça azalmaktadır. Özellikle entübasyonun ilk günlerinde, COVID-19 enfeksiyonuna sekonder VİP görülme sıklığını azaltmak için pron pozisyonundaki hastaların ağız bakımı gibi VİP önlemlerinde yer alan bakım yaklaşımların sürdürülmesi önemlidir.

Anahtar Kelimeler: Pron; supin; ventilatörle ilişkili pnömoni; akut solunumsal stres sendromu; COVID-19

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Ventilator-associated pneumonia (VAP) is a pneumonia that develops in patients undergoing invasive mechanical ventilation (MV) for at least 48 hrs.[1] VAP is one of the most important hospital-acquired infection in the intensive care unit (ICU). It is stated that the VAP incidence is 10%-50%, and is closely related to longer ICU stays, and more hospitalization costs higher mortality.[2]

Position is an important preventive strategy to decrease the VAP prevalence, including the supine, semi-recumbent, and prone positions (PPs) among mechanically ventilated patient. The prone position is a frequently used approach in acute respiratory distress syndrome (ARDS) patients hospitalized in the ICU to improve ventilation/perfusion ratio and gas exchange and reduce the risk of VAP.[3,4] However, there are controversial results in studies that studied the effects of PP on VAP. It was reported that the PP could decrease the risk of VAP in intubated patients compared with the supine position since PP limits gastroesophageal reflux and enables drainage of airway secretion.[5] However, a study found that the incidence of VAP was lower in PP patients than supine supine-position patients, other meta-analysis studies found that prone did not affect VAP incidence.[2,6] It is suggested that PP should be maintained for more than 16 hours to provide alveolar recruitment efficiently.[7] However, Zhu et al. highlighted that if the daily PP time was less than 16 hours, the incidence of VAP had a tendency to decrease, and the all-cause mortality reduced significantly in the daily PP time ≥16 hours.[2] However, it was recommended that prone positioning for more than 12 hours for moderate and severe coronavirus disease-2019 (COVID-19), intubated patients with PaO_2/FiO_2<150 in the "Sepsis Survival Campaign 2019 in Coronavirus-Associated Critical Patients" guideline.[8]

The daily prone time threshold at which VAP development is prevented, but the benefit of PP must also be obtained should be determined for mechanically ventilated patients.[2] Zhu et al. highlighted that due to inadequate evidence, it is difficult to know whether a specific period and duration of PP can reduce the incidence of VAP without reducing survival.[2] The effect of PP on VAP is controversial, and it has always been an important issue for researchers in the intensive care field. Centers for Disease Control and Prevention (CDC) reported that VAP incidences in ICUs increased by 11-44% during the COVID-19 pandemic.[9] Therefore, this study aimed to determine the effect of position on the development of VAP in COVID-19 patients in an ICU.

MATERIAL AND METHODS

This retrospective and case-control study was conducted as a cross-sectional and descriptive study using the Strengthening the reporting of observational studies in epidemiology statement (STROBE) statement.

SAMPLE SIZE

The study population consisted of 600 intubated adult patients admitted to the surgical and medical ICU of a Koç University hospital between March 2021 and March 2022. The study sample consisted of 138 patients over 18 years of age, admitted to the ICU due to COVID-19, and intubated for at least 48 hours. The power analysis was conduct. The sample size was calculated with the G*Power 3.1 program by. According to the results of the regression analysis in the prone positioned group, a total of 59 samples, 5 estimators. If the R^2 value is 56%, the minimum number of samples was found to be 51 at the 95% confidence and power level.

DATA COLLECTION

In the study, data collection form developed by the researchers considering the studies in the literature were used to collect data. Demographic and clinical data were obtained from electronic patient records.

Data on demographic characteristics and clinical characteristics of the patient including age, gender, and comorbid diseases, the diagnosis for admission to the ICU, the duration of intubation, the development of VAP, pathogens of VAP, peptic ulcer prophylaxis, Richmond Sedation and Agitation Score (RASS), and The Acute Physiology and Chronic Health Evaluation (APACHE) II score were obtained. In addition, the following data were also obtained: Duration of MV (day), the day of the development of VAP after intubation, the pathogens of VAP, length of stay in ICU (day), the day the PP started after in-

tubation, frequency of prone positioning (times) and daily prone time (hours) and survival. Pneumonia is considered ventilator-associated if the clinical, laboratory, imaging, and microbiological findings occur in patients receiving MV for at least 48 hours. In the ICU where the study was conducted, treatment and nursing care interventions were carried out in line with the VAP bundle recommended by CDC.[10]

VAP was diagnosed based on radiological, clinical and laboratory findings.[11] Accordingly, the presence of at least one of the findings of progressive infiltration, consolidation and cavitation in 2 or more consecutive chest radiographs, in the clinical and laboratory findings the presence of at least one of the findings of fever (≥38C), leukocytosis, leukopenia and mental status change that cannot be explained by another reason for those over the age of >70, and the presence of at least 2 of the findings of purulent sputum, cough, dyspnea or tachypnea, bronchial sound deviation, deterioration in blood gas values and growth in the lower respiratory tract sample were taken into consideration and VAP was diagnosed in patients.[11]

STATISTICAL ANALYSIS

The data were analyzed using the SPSS software, version 26.0 (IBM, USA) for Windows. The descriptive statistical methods used numbers, percentages, means, medians, and standard deviation. The continuous data on the demographic and clinical characteristics of the patient showed a normal distribution. The significance was evaluated at $p<0.05$.

Factors Affecting VAP development among patients with PPs were evaluated by the logistic regression using the backward model. Factors included hypertension (HT), other comorbid diseases, smoking, RASS score, APACHE score, duration of intubation, duration of enteral nutrition, duration of daily PP, and total duration of PP during ICU stays.

ETHICS APPROVAL AND CONSENT

The study was approved by the Koç University Institutional Review Board (date: June 2, 2022, no: 2022.168.IRB2.030). This study was conducted in accordance with the Principles of the Declaration of Helsinki. Verbal and written consent was obtained from the patients' families.

RESULTS

A total of 138 patients were included in the study. Majority of patients was male in both supine and PP groups. Mean age was 66.23 (±15.41) in PP and 69.05 (±12.39) in supine position ($p>0.05$). It was determined that the patients in the PP were intubated for 15.30±10.83 days, and in the SP group were intubated for a total of 14.72±12.47 days ($p<0.05$) (Table 1). The VAP incidence was 52.5% in PP group and 21.5% in supine position group ($p<0.05$). It was determined that the PP group was started 3 (±5.8) days after the intubation and remained in each prone for an average of 17.19 (±12.09) hrs (Table 2).

The results regarding the multivariate regression findings are given in Table 3. In the logistic regression analysis, the variables that most often cause VAP development were being in the PP, having HT, and other comorbid diseases, respectively ($p<0.05$) (Table 3). Additionally, affecting factors for the development of VAP among only patients in PP included duration of MV, smoking, and having other comorbid diseases. In other words, the higher incidence of VAP was due to prolonged MV time, smoking and having comorbid conditions. Furthermore, it was found that the increased duration of the daily PP was the negatively affecting factor for the development of VAP ($p<0.05$) (Table 3).

DISCUSSION

The multiple regression analysis showed that prone positioning, HT, and other comorbid diseases significantly affected the development of VAP among intubated COVID-19 patients.

In this study, PP was one of the most effecting factors on VAP development among COVID-19 patients in regression analysis. Controversial findings stated in studies that determined the effects of position on VAP development among non-COVID-19 patients. While a study concluded that PP was associated with a lessening in VAP incidence in ARDS patients, other recent meta-analysis studies found that prone did not affect VAP incidence.[2,6,12] This study found that the PP was the factor that most affected the development of VAP. However, during the

TABLE 1: Comparison of demographic and clinical characteristics of the patients with prone and supine position (n=138)

	PP (n=59)	Supine position (n=79)		
Demographic and clinical characteristics	**n**	**n**	χ^2	**p value**
Gender				
Man	36 (61%)	51 (64.5%)	0.18	0.67
Woman	23 (39%)	28 (35.5%)		
HT				
No	32 (54%)	46 (58%)	0.21	0.64
Yes	27 (46%)	33 (42%)		
Diabetes mellitus				
No	39 (66%)	54 (68%)	0.07	0.78
Yes	20 (34%)	25 (32%)		
Hyperlipidemia				
No	58 (98%)	75 (94.9%)	1.09	0.29
Yes	1 (2%)	4 (5.1%)		
Obesity				
No	58 (98%)	78 (98.7%)	0.75	0.38
Yes	1 (2%)	1 (1.3%)		
Other comorbid diseases				
No	29 (49.1%)	23 (38.9%)	5.77	**0.01**
Yes	30 (59.9%)	56 (61.1%)		
Smoking				
No	44 (74.5%)	58 (73.4%)	0.02	0.87
Yes	15 (25.5%)	21 (26.6 %)		
Peptic ulcer prophylaxis				
No	1 (2%)	5 (6.3%)	1.74	0.18
Yes	58 (98%)	74 (93.7%)		
RASS				
-5	0 (0%)	2 (2.5%)	8.91	0.25
-4	49 (83%)	54 (68%)		
-3	3 (5 %)	7 (9%)		
-2	3 (5 %)	1 (1%)		
-1	1 (2%)	3 (5%)		
0	1 (2%)	2 (2.5%)		
1	2 (3 %)	9 (11%)		
3	0 (0%)	1 (1%)		
Ventilator associated pneumonia (VAP)				
No	28 (47.5 %)	62 (78.5 %)	14.33	**<0.001**
Yes	31 (52.5 %)	17 (21.5 %)		
Pathogens of VAP				
Acinetobacter baumanni	19 (61.2%)	4 (23.4%)		
Psedomonas aeruginosa	4 (12.8%)	6 (35.2%)		
Escherichia coli	1 (3.2 %)	0 (0%)		
Enterobacter cloacae	0	1 (5.8%)	9.94	0.192
Clebsiella pneumoniae	4 (12.8%)	4 (23.4%)		
Methicillin resistant staphylococcus aureus	1(3.2 %)	1 (5.8%)		
Streptococcus pneumoniae	1(3.2 %)	0 (0%)		
Stenotrophomonas maltophilia	1(3.2 %)	1 (5.8%)		
Mortality				
Yes	52 (88.2%)	47 (59.5%)	13.66	**<0.001**
No	7 (11.8%)	32 (40.5%)		

TABLE 1: Comparison of demographic and clinical characteristics of the patients with prone and supine position (n=138) *(contunied)*.

	PP (n=59)		Supine Position (n=79)			
	$\bar{X}$	SD±	$\bar{X}$	SD±	t value	p value*
Age	66.23	15.41	69.05	12.39	1.19	0.42
APACHE II score	23.2	6.1	19.7	9.1	1.4	0.31
Duration of intubation (days)	15.3	10.83	14.72	12.47	-0.29	0.87
Duration of enteral nutrition (days)	14.1	10.2	13.91	12.06	-0.12	0.38
Length of stay in ICU (days)	18.8	11.08	17.65	11.4	-0.636	0.69
The day of the VAP development after intubation	9.23	5.98	11.52	10.6	1.35	**0.002**

*p<0.05; PP: Prone position; SD: Standard deviation; χ^2: chi-square test; t: Independent Samples t-test; RASS: Richmond Agitation and Sedation Score; CRP: C reactive protein; APACHE: Acute Physiology and Chronic Health Evaluation; ICU: Intensive care unit; VAP: Ventilator associated pneumonia

TABLE 2: Data of patients with prone position (n=59)

	PP Patients (n=59)		
Data of patients with prone position	$\bar{X}$	SD±	Minimum-maximum
The day the prone position started after intubation	3.04	5.8	0-24
Total number of PPs given during ICU stay (times)	6.10	5.10	1-20
Duration of daily prone position (hours)	17.19	12.09	9-22

PP: Prone position; SD: Standard deviation; X: Mean; ICU: Intensive care unit

TABLE 3: Factors affecting VAP development among all patients (n=138) and among patients with prone position (n=59)*

							95% CI for EXP (B)	
Factors Affecting VAP Development	**B**	**SE**	**Wald**	**df**	**Sig.**	**Exp (B)**	**Lower**	**Upper**
Constant	-0.984	0.337	8.527	1	0.004	0.374		
Type of position	1.364	0.414	10.856	1	**<0.001**	3.913	1.738	8.809
Duration of mechanical ventilation	-0.016	0.040	0.166	1	0.683	0.984	0.909	1.064
Duration of enteral nutrition	0.053	0.040	1.697	1	0.193	1.054	0.974	1.141
HT	-1.134	0.421	7.271	1	**0.007**	0.322	0.141	0.734
Comorbid diseases	1.022	0.424	5.820	1	**0.016**	2.779	1.211	6.374
							95% CI for EXP (B)	
Patients with prone position	**B**	**SE**	**Wald**	**df**	**Sig.**	**Exp (B)**	**Lower**	**Upper**
Constant	5.381	2.275	5.594	1	0.018	217.168		
Duration of mechanical ventilation	0.138	0.046	8.873	1	**0.003**	1.148	1.048	1.257
Smoking	2.097	0.938	5.005	1	**0.025**	8.146	1.297	51.161
Comorbid diseases	1.860	0.769	5.854	1	**0.016**	0.156	0.034	0.702
Duration of daily prone position	-0.376	0.134	7.913	1	**0.005**	0.687	0.528	0.892
Total duration of prone position during ICU stays	-0.006	0.006	0.848	1	0.357	0.994	0.982	1.006

*R^2: 56%; VAP: Ventilator-associated pneumonia; SE: Standard error; Wald: Chi-square test; df: degree of freedom; Sig.: Significance; ICU: Intensive care unit; CI: Confidence interval

COVID-19 pandemic, the most crucial factor leading to the higher incidence of VAP in this study may be attributed to the paucity of nursing staff may have resulted in interruptions in patient care. In addition, the result obtained in this study showed that VAP, which is developed in the early period in PP due to *A Acinetobacter baumannii* (*A. baumannii*), has developed in patients secondary to COVID-19 infection. A prospective study found that *Acinetobacter* species, *P. aeruginosa*, *S. aureus*, and *K. pneumonia* were the causes of VAP. In COVID-19 patient, the secondary infections development rate was between 45% and 91.2% caused by *A. baumannii*.[13,14] In various studies, it has been shown that *A. baumannii* infection in

COVID-19 patients increases the mortality rate, length of stay in the ICU, and the need for MV support.[15,16] Secondly, hand hygiene, contact precautions, correct use of personal protective equipment, environmental cleaning, and disinfection have proven effective in managing *A. baumannii* infection in the ICU during COVID-19.[17] In this study, the high rate of VAP in prone patients due to *A. baumannii* may have also been caused by situations such as the decrease in the frequency of oral care due to the high contagiousness of the coronavirus, the misuse of parapneumonic effusion (PPE), inadequate hand hygiene and aseptic technique applications, the lack of training on cleaning and disinfection, the high circulation of healthcare providers and excessive workload during COVID-19 pandemic.

This study determined that HT was also an affecting factor of VAP development in COVID-19 patients. Although there are conflicting opinions on HT, a meta-analysis and meta-regression study also stated that HT is associated with worse patient outcomes, increased mortality, and a greater need for intensive care in COVID-19 patients.[18] It is known that severe COVID-19 causes significant vascular abnormalities, including macro- and micro-vascular coagulopathy and thromboembolism, and renal and cardiac failure. It was stated that there is an association between HT and poor outcomes in severe COVID-19 patients due to microvascular effect.[19] The result obtained from this study suggests that HT as an independent factor associated with VAP development may be explained by a worse tolerance of hemodynamic impairment with more organ dysfunction in COVID-19 patients compared with other patients.

The higher incidence of VAP in our study was also due to comorbid conditions. The risk of death from COVID-19 is strongly related to the previous health of the patient.[20] It is specified that chronic comorbidities, such as cardiovascular disease and pulmonary disease, increase the incidence of fatal outcomes.[21,22] Many of the older patients who become severely ill often die with COVID-19 but are highly frail and the presence of comorbid conditions causing immunosuppression/infection during the stay in ICU.[23,24] It is known that patients who develop VAP are more likely to suffer from conditions that cause immunosuppression, such as chronic renal failure, diabetes mellitus, and steroid therapy. Considering that most of the patients in this study had cardiovascular and pulmonary diseases other than HT, it is thought that these comorbid diseases predispose to the development of VAP due to multiple organ failure or the immunosuppressive effects of the treatments and supportive treatments provided to the patients.

This study also determined the effecting factors for VAP among COVID-19 patients with PPs, including smoking, intubation duration, daily PP, and comorbid diseases other than HT.

Smoking was a significant risk factor for VAP development among prone-positioned patients in this study. The meta-analysis study determined that smoking increased the risk for development of severe COVID-19.[14] Zhang et al. specified that smoking was associated with critical outcomes and increased risk of ICU admission and mortality in COVID-19 patients.[25] Studies found that smoking increases the VAP development risk, because smoking deteriorates mucociliary clearance, making it easier for microorganisms to colonize in the respiratory tract.[14,26,27] Cigarette smoke has different compounds, which adversely affect respiratory tract cells. It is stated that the effect of smoking on the immune system may vary between smoking patients and passively exposed ones.[28] Smoking can lead to oxidative stress and lung inflammation, making smokers more vulnerable to bacterial or viral infections. Similar to previous studies, results obtained in this study showed that smoking, due to its effects, makes COVID-19 patients more susceptible to developing VAP and worsening patient outcomes.

In this study, it was found that the duration of intubation affected the development of VAP. Furthermore, the patients in the PP were intubated for 15 days, and VAP developed on the ninth day of intubation. In their meta-analysis study, Zhu et al. highlighted that it is difficult to observe a specific period that can decrease the incidence of VAP without increasing mortality.[2] Intubation may cause a lack of saliva which is related to side effects from the multiple medications that patients receive and prolonged

mouth opening due to endotracheal tube (ETT). Regular oral care with chlorhexidine gluconate (CHG) provided by ICU nurses acts like saliva by moistening the oral mucosa and removing plaque. Randomized controlled trial (RCT)s specified that oral care with chlorhexidine before intubation may significantly eliminate the risk of expending microorganisms from the oral cavity into the lungs during the intubation.[29,30] The risk for VAP development increases with longer intubation time may show that there may be disruptions in providing regular oral care in patients who remain in the PP for a long time. Although it is known that it is one of the interventions that cause aerosol dissemination, it is known that oral care provided in patients at regular intervals reduces the incidence of VAP. Therefore, nurses should obey VAP bundle activities using appropriate PPE. Furthermore, endotracheal intubation usually proceeds without any preparation. ICU nurses can consider oral care before intubation and incorporate it into their routine clinical practice to decrease VAP rates in mechanically ventilated patients in a prolonged PP. Decreasing the bacterial burden from the oral cavity with regular oral care would reduce the possibility of bacteria being aspirated into the lung and significantly decrease the VAP rate. Therefore, it is recommended that more emphasis should be placed on treatment and care interventions included in VAP bundles in the first week after intubation, where there is an increased risk of VAP development.

In this study, it was found that the VAP incidence decreased as the prone time increased. Studies suggested that PP should be maintained for 16 hrs or more to affect alveolar recruitment positively.[31] In meta-analysis studies, prone was the most effective for reducing the mortality risk, but it did not affect VAP incidence.[2,12] Prone positioning is recommended in patients with severe ARDS for more than 12 hrs per day by several guidelines.[8,32] The PP has been widely used in patients with ARDS to improve gas exchange include alveolar recruitment, arterial oxygenation, and respiratory compliance by expanding the posterior alveoli to maintain a better ventilation/perfusion ratio and postural drainage of secretions.[33] While a study concluded that PP was associated with a decrease in VAP in ARDS patients, Zhu et al. highlighted that when the duration of daily PP was less than 16 hours, the incidence of VAP tended to decrease.[2] Homogenizing the tidal volume distribution can decrease the stress of tissue and the detrimental effects of MV. Although this study and other studies found that the PP increased the incidence of VAP, the duration of the PP decreased the VAP incidence. The results show that improved oxygenation could provide time for lung healing processes and reduce secondary lung infection or trauma, recovery was accelerated. Meta-analysis studies concluded that subglottic secretion drainage effectively reduced the VAP rate.[34] Therefore, in patients for whom a prolonged PP is inevitable, ICU specialists can consider using endotracheal tubes that allow subglottic secretion aspiration intermittently and integrate it into VAP preventive strategies in ARDS patients.

LIMITATIONS

Since the intensivists in the ICU made the PP decision, they selected the patients who could tolerate the prolonged PP.

CONCLUSION

The incidence of VAP was high in intubated COVID-19 patients with HT and in the PP. Furthermore, smoking, duration of intubation, and other comorbid diseases increased the risk of VAP development. Nurses should emphasize hand hygiene and aseptic technique applications, environmental cleaning and disinfection, and meticulously maintaining care approaches such as regular and frequent oral care of patients in PPs to reduce the incidence of VAP secondary to COVID-19 infection, especially during the first week after intubation.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or mem-

bers of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

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