ORIGINAL ARTICLE

Evaluation of the new modified apnea test in confirmation of brain death

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Background: Apnea testing is mandatory to confirm brain death; however, it is unsafe for patients who have substantial hypoxemia without ventilator support. We used a new modified apnea test without the need to disconnect the patient from the ventilator in the present study and compared the outcomes and complications of the new method to the widely used old method. **Materials and Methods:** The current study was conducted on people suspected of having brain death. Both the old and new apnea tests were carried out on the same individual. In the new modified method, instead of hyperventilating and then separating the brain death from the ventilator, the induced hypercapnia method was used, and instead of performing repeated arterial blood gas (ABG), the target $ETCO_2$ was obtained, and at the time of the target $ETCO_2$, ABG was also checked followed by comparing $ETCO_2$ with $PaCO_2$. **Results:** Thirty patients, including 25 (83.3%) males and 5 (16.75%) females, were included in the study. The results showed significant improvement in terms of O_2 saturation and heart rate (HR) using the new modified apnea test compared to the common test. Systolic blood pressure, diastolic blood pressure, and the frequency of complications were improved in the new modified test. **Conclusion:** The modified apnea test produced better results in terms of O_2 saturation, HR, and other clinical factors, while it does not require disconnection from the ventilator and repeated ABG assessment. Therefore, it can be used to successfully diagnose brain death in high-risk individuals suffering from severe hypoxia.

Key words: Apnea test, arterial blood gas, brain death, hypoxia

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INTRODUCTION

Today, organ transplantation, as the final treatment for the failure of many organs, plays a critical role in ensuring societal health and improving the quality of life. It is well known that reducing disease complications and treatment costs is possible, and the most important source for organ donation is brain-dead patients, from whom approximately 90% of transplanted organs are obtained.[1,2] However, the definition of death, and particularly brain death, is a significant problem in organ transplantation.[2] Brain death is defined as the permanent and irreversible cessation of all the actions of all parts of the brain, including the cortex, subcortex, and brain stem at the same time. Necessary criteria for brain death include: (a) lack of activities of the cerebral hemispheres; (b) lack of brainstem activity; and (c) the irreversibility of the mentioned conditions.[3-5] There are many tests and evaluations to confirm brain death, and the apnea test is one of them that is crucial for clinical and legal confirmation. By providing the patient with a hypercarbic respiratory stimulus, the apnea test checks to see if the medullary function has been lost or preserved. The ensuing hypercarbia causes cerebral spinal fluid acidosis, which in turn activates the medulla's respiratory reflex.[6] If no respiratory activity is observed, the apnea test confirms brain death and is a sign of complete brain stem inactivity;[7] however, if hypoxia develops during the test, the test should be terminated. On the other hand, patients who have been seriously injured and have underlying diseases that

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make respiratory and hemodynamic stabilization difficult are at a higher risk than the general population. As a result, apnea testing is generally avoided in these patients for fear of worsening their hemodynamic and respiratory dysfunction, perhaps leading to cardiac arrest.[7]

Given the risks of disconnecting the brain-dead patient from the ventilator, as well as the possibility of hypoxia and arrhythmia, and the difficulty of performing repeated arterial blood gas (ABG) tests, a new method is used in this study. The goal of this study is to compare the efficiency of this new method in confirming brain death to the common apnea test, as well as the complications involved.

METHODS

The current cross-sectional study, conducted in 2018 and 2019 at Al-Zahra Hospital in Isfahan, included all patients suspected of brain death during this period. The study was registered with the registration number IRCT20200209046433N1. Both the old and new apnea tests were performed on the same person. Before performing the test, an ABG was taken and a capnograph was used, and all conditions of the standard capnography were followed. The difference between ABG and capnograph was measured before and after the test. During the test, the patient's spontaneous breathing, hemodynamics, and oxygenation were monitored. In the selection of cases, it was preferable to examine the healthier individuals without pneumonia, *acute respiratory distress syndrome*, and fever. In the studied subjects, the order of performing the tests was changed one by one. For example, in person 1, the new test was performed first, then the old test, and vice versa in the second person. The entrance conditions for the two tests were the same, and it was at least 1 h. Obtaining consent to participate in the study was considered an inclusion criterion, and hemodynamically unstable conditions of subjects were considered an exclusion criterion. Necessary conditions for performing the test were a body temperature of more than 36°C, arterial systolic blood pressure (SBP) of more than 90 mmHg, arterial carbon dioxide pressure at a normal level (35–45 mmHg), arterial oxygen pressure of more than 200 mmHg, and no sign of electrolyte disorder.[8-10]

Procedure of common apnea test

Before disconnecting the patient from the artificial respiration device (ventilator), 100% oxygen was administered to the patient for 10 min, after which the patient was disconnected from the device and oxygen was administered at a rate of 6 L/min through a narrow Nelaton catheter inserted into the tracheal tube. Serial gasometry was used to gradually raise the $PaCO_2$ – 60 mmHg or 20 mmHg above the initial baseline level. If no respiratory activity was detected, brain death and a sign of complete inactivity of the brain stem were confirmed.

Procedure of new modified apnea test

In the new modified method, instead of hyperventilating and then separating the brain death from the ventilator, the induced hypercapnia method was used to increase the $PaCO_2$ with the smallest minute volume (10% determined volume) that can be set on the ventilator. In our study, the ventilator mode was changed to synchronized intermittent mechanical ventilation (SIMV) with set rate 4, and instead of performing repeated ABG, we obtained the target $PaCO_2$ using $ETCO_2$, and at the time of the target $ETCO_2$, ABG was also checked followed by comparing $ETCO_2$ with $PaCO_2$.

Statistical analysis

Statistical analyses were done using SPSS software for Windows (SPSS, Inc., Chicago, IL, USA, version 25). Data were reported as mean ± standard deviation or median (interquartile range) and number (percent) as appropriate. The paired *t*-test and McNemar test were used to compare all variables between the two methods. The change of quantitative variables over time within each group was assessed using repeated measurements of ANOVA. The level of significance is considered to be <0.05.

RESULTS

Finally, 30 patients were included in our study. The demographic and basic factors of participants are listed in Table 1. As shown in the table, the leading causes of brain death in the study population were stroke (46.7%) and *intracerebral hemorrhage* (16.7%).

Table 1: Demographic and basic characteristics of study population

Characteristics	Values
Age (year)	36.9±13.4
Gender	
Male	25 (83.3)
Female	5 (16.7)
Clinical factors	
Tidal volume	504.48±91.09
Pressure support	11.76±2.29
Respiratory rate	11.97±2.08
PEEP	5.45±1.02
FiO_2	0.46±0.08
Cause of hospitalization	
ICH	5 (16.7)
Stroke	14 (46.7)
Tumor	4 (13.3)
Head injury	4 (13.3)
Suicide	3 (10)

Data presented as mean±SD or *n* (%). SD=Standard deviation; PEEP=Positive end-expiratory pressure; ICH=Intracerebral hemorrhage

The history of underlying diseases, complications, and clinical conditions of participants in each group (common Apnea test vs. new modified Apnea test) are listed in Table 2, and as data showed, there were no significant differences between these two groups in terms of each variable.

Table 3 shows the comparison of clinical factors, including SBP, diastolic blood pressure (DBP), heart rate (HR), and O_2 saturation at each time (0 until 10) between the two methods. As can be concluded from the table, there is a significant difference between the two methods in terms of HR and O_2 saturation at some time points.

Figures 1 and 2 show that over time, the averages of SBP from time 3 onward and DBP from time 5 onward have been increasing in the normal range, and SBP and DBP were improved in the new method, although the difference is not statistically significant.

Table 2: Incidence of complications in new method and old methods

Complications	New method (*n*=30)	Old method (*n*=30)	*P*
Arrhythmia	0	2 (6.7)	<0.001
Cardiac arrest	0	0	-
Hypertension	1 (3.3)	5 (16.7)	<0.001
Tachycardia	4 (13.3)	10 (33.3)	0.011
Bradycardia	0	1 (3.3)	<0.001
Hypoxia	0	3 (10)	<0.001
Stop apnea test	0	1 (3.3)	<0.001
Other complications (pneumothorax)	0	0	-

Data are presented as *n* (%). *P* values calculated by McNemar's test

Figure 3 shows that the HR mean increases in both methods, with the old method increasing faster than the new method after 4 min. The O_2 saturation chart [Figure 4] shows a decreasing trend over time, which was more prevalent in the old method.

Table 4 shows the comparison of Base excess (BE), $PaCO_2$, and PaO_2 variables in two apnea test methods at the first time and the last time (after 10 min). Based on the results, there was no significant difference in BE and $PaCO_2$ variables at the first time and last time. However, PaO_2 showed a significant difference in the last time which confirmed better results for PaO_2 using the new modified apnea test. Based on $PaCO_2$, both methods confirmed the brain death successfully.

DISCUSSION

Testing for apnea has always been a crucial step in identifying brain death. Our results showed significant improvement in terms of O_2 saturation and heart rate using a new modified apnea test compared to the old common method. All complications were improved and significantly reduced using the new modified test. Our results in line with the findings of other researchers showed that testing for apnea while the patient was still connected to the ventilator was safer than disconnecting them.[10]

One of the purposes of the apnea test is to induce respiratory acidosis by raising CO_2, which is considered a respiratory stimulation mechanism; however, hypoxia during the test is a problem that should result in the test being stopped. This is one of the most hazardous and well-known side effects

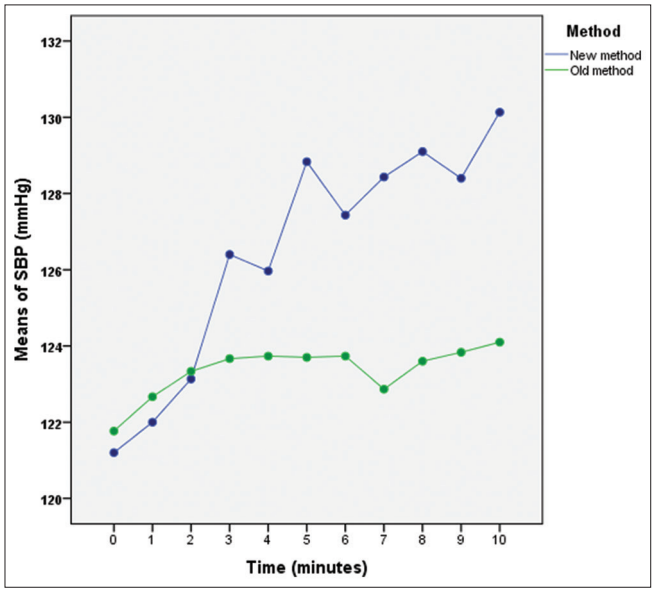

Figure 1: Comparison of systolic blood pressure mean between old and new methods during 10 min (repeated measures ANOVA; *P* = 0.306). SBP = Systolic blood pressure

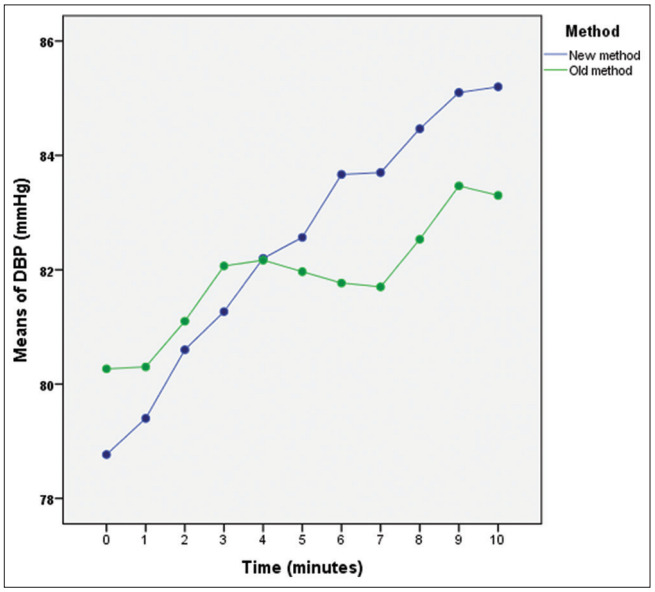

Figure 2: Comparison of diastolic blood pressure mean between old and new methods during 10 min (repeated measures ANOVA; *P* = 0.705). DBP = Diastolic blood pressure

Table 3: Comparison of clinical factors in each min (0 until 10) between two methods

Clinical factors	Methods	Time (min)										P_{Time}	P_{Method}
		0	1	2	3	4	5	6	7	8	9	10	
SBP	New	121.2±10.2	122.0±9.5	123.1±8.8	126.4±11.3	126.0±7.0	128.8±14.6	127.4±8.6	128.4±9.3	129.1±11.0	128.4±13.2	130.1±15.6	0.022	0.306
	Old	121.8±6.6	122.6±6.5	123.3±7.2	123.6±9.1	123.7±11.5	123.7±14.4	123.7±17.5	122.8±21.2	123.6±23.5	123.8±27.0	124.1±30.3	0.761	
	P^a	0.674	0.585	0.897	0.217	0.320	0.115	0.254	0.146	0.188	0.361	0.251		
DBP	New	78.7±6.8	79.4±5.7	80.6±4.8	81.2±4.2	82.2±4.1	82.5±4.5	83.6±5.6	83.7±5.8	84.5±6.4	85.1±6.8	85.2±8.1	0.006	0.705
	Old	80.2±5.1	80.3±5.2	81.1±4.5	82.1±5.3	82.1±6.6	81.9±7.5	81.7±8.6	81.7±10.1	82.5±11.9	83.4±13.2	83.3±15.3	0.369	
	P^a	0.201	0.431	0.656	0.511	0.981	0.722	0.321	0.346	0.406	0.484	0.503		
HR	New	80.4±8.8	80.8±8.4	81.3±8.4	82.3±8.2	82.2±8.7	83.0±9.0	84.0±8.9	85.2±9.3	87.4±8.8	88.5±9.7	90.0±11.2	<0.001	0.119
	Old	78.0±6.8	78.9±6.6	80.7±6.4	82.2±6.2	84.1±5.7	85.6±5.0	85.0±16.4	90.3±5.7	93.5±5.5	96.8±6.5	101.4±8.1	<0.001	
	P^a	0.010	0.041	0.478	0.903	0.165	0.054	0.799	0.002	<0.001	<0.001	<0.001		
O_2 saturation	New	96.4±8.4	99.1±2.5	98.2±1.5	97.3±1.4	96.4±1.5	95.2±1.7	94.4±1.8	92.7±5.8	92.7±2.1	91.9±2.4	90.3±6.0	<0.001	<0.0001
	Old	99.2±0.8	98.2±0.9	96.9±1.1	95.3±1.5	93.8±1.9	92.6±1.8	91.1±1.9	89.6±1.6	88.0±1.8	86.7±1.7	85.9±2.2	<0.001	
	P^a	0.081	0.034	<0.001	<0.001	<0.001	<0.001	<0.001	0.009	<0.001	<0.001	0.001		

[a]*P*-value calculated by a paired *t*-test. Data presented as mean±SD. SD=Standard deviation; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; HR=Heart rate

of an apnea test. To avoid such a dangerous complication, researchers used an alternative method that included exogenous carbon dioxide and $EtCO_2$ was calculated. There was an unnoticeable decrease in arterial blood pressure with the method described by these researchers, but no other side effects were observed. As a result, the authors regard this method as a reliable and safe alternative to the apnea test.[11] Park *et al*. used a modified apnea test for confirming brain death.[12] In their modified method after disconnection from the mechanical ventilator, 100% oxygen was delivered through the endotracheal tube connected to the Ambu bag with positive end-expiratory pressure (PEEP). According to their results, there were no significant differences in terms of $PaCO_2$, PaO_2, or pH between the two groups (conventional apnea test vs. modified test); however, the modified method prevented dramatic reductions in PaO_2 in overweight subjects and those who had hypoxic brain injury due to hanging.[12]

Ahlawat *et al*. introduced a new modified apnea test without the removal of the ventilator by lowering minute ventilation (MV) by at least 50% relative to stabilized baseline ventilator settings. Their justification for lowering MV by about 50% is that brain-dead patients frequently have hemodynamic instability in addition to hypoxia. Hemodynamic instability may get worse if the MV varies rapidly. When someone is hypoventilating, their $PaCO_2$ level gradually rises, and periodic ABG can detect a $PaCO_2$ level that is 20 mmHg higher than normal. In their study, the ventilator mode was changed from volume control to CPAP once the goal $PaCO_2$ level was reached to maintain the airways by sustained PEEP administration and increase the likelihood of sufficient alveolar oxygenation. After that, the patient was watched for 60 s to check for indications of

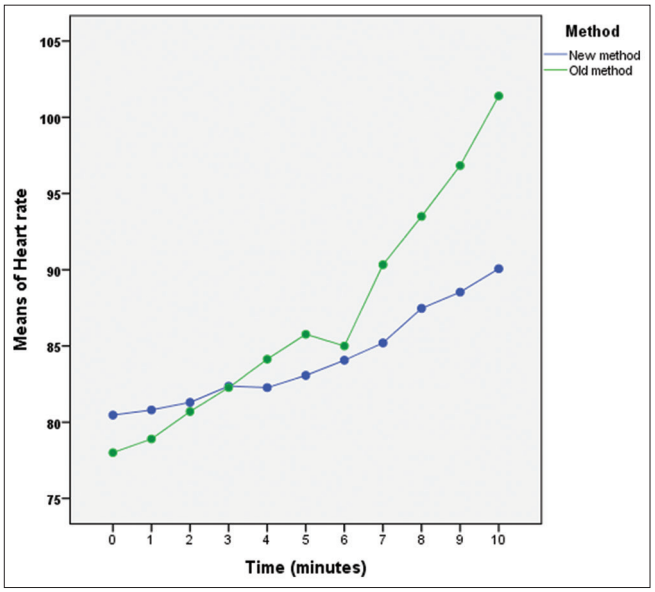

Figure 3: Comparison of heart rate mean between old and new methods during 10 min (repeated measures ANOVA; $P = 0.119$). HR = Heart rate

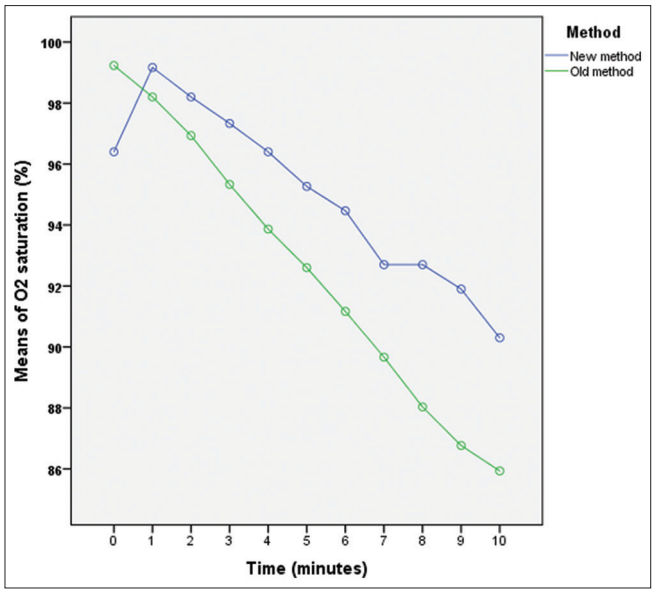

Figure 4: Comparison of O_2 saturation mean between old and new methods during 10 min (repeated measures ANOVA; $P < 0.0001$)

breathing. The patient is classified as apneic if there is no evidence of breathing effort.[13]

In a study conducted over 3 years from 2011 to 2014, 57 brain-dead patients were selected using an easy sample method. Apnea testing was performed on all individuals. After preoxygenation and meeting the test conditions, the patients were removed from the ventilator for 10 min, and ABGs were measured four times: at 8, 5, 2, and 10 min. The test was declared positive if the arterial carbon dioxide level exceeded 60 mmHg or increased by more than 20 mmHg from the baseline value; the test was terminated if any of the predefined problems occurred. According to the findings of this study, 11 persons experienced difficulties throughout the test. Ten individuals experienced severe hypoxia and one experienced cardiac arrest. Complications occurred in 85% of patients who had adverse pretest status, while this rate was around 10% in other cases. The average duration between the announcement of death and the completion of the apnea test ranged from 36 h to 11 days. In all of these cases, the test results were positive. This study concluded that the apnea test is an intrusive technique that may deplete organ reserve for transplantation and cause tissue ischemia due to potentially severe cardiovascular consequences.[14] They reported that their modified method not only could be a safe option for suspected brain-dead patients to be checked without risk of worsening the hypotension and hypoxia but also can prevent serious cardiovascular consequences and damage to the organ intended for transplantation.

CONCLUSION

The new modified apnea test used in our study produced significantly better results in terms of HR and O_2 saturation, indicating that it can be used to safely screen for apnea in complicated patients who need to be tested for brain death, like those who have substantial hypoxia or hypotension that is only mildly responding to hemodynamic therapy. SBP and DBP also were improved but not significantly which could be due to the low number of subjects in our study.

Table 4: Compassion of clinical factors in the first and last times between two methods

Variables	Methods	First time	Last time	*P*
BE	New	−1.6±3.8	−0.91±3.9	0.034
	Old	−1.7±2.9	−1.08±3.9	0.152
	P	0.939	0.87	
$PaCO_2$	New	39.8±3.6	66.2±4.1	<0.001
	Old	40.4±2.9	65.1±4.4	<0.001
	P	0.506	0.335	
PaO_2	New	233.3±30.2	95.7±16.9	<0.001
	Old	233.8±28.3	75.5±8.0	<0.001
	P	0.939	<0.001	

Data presented as mean±SD. *P* values calculated by paired *t*-test. SD=Standard deviation; BE=Base Excess

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Conflicts of interest
There are no conflicts of interest.

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