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Sensitivity, specificity and cut-off point of the Montreal Cognitive Assessment (MoCA) in Patients with mild-Traumatic Brain Injury (mTBI)

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Abstract

Background: Although patients with mild traumatic brain injury (mTBI) rarely exhibit an identifiable lesion on neuroimaging, they frequently experience neurocognitive problems.

Objectives: The present study aimed to determine the cut-off point, sensitivity, and specificity of the Montreal Cognitive Assessment (MoCA) test in mTBI patients.

Methods: In this cross-sectional-analytical study, the case group included 79 patients with mTBI were enrolled in the trauma, neurosurgery, and ICU ward of PourSina hospital (northern Iran), and there were 79 healthy individuals in the control group. Both groups were participating in this study were cognitively evaluated by the MoCA and MMSE test. Moreover, as retesting reliability and determining the concurrent and convergent validity of the MoCA, and Pearson correlation coefficient between two groups, MMSE test was performed on 20 mTBI patients with an average time interval of 3 days. The independent t-test, Cronbach's alpha and discriminant analysis used for determining the distribution, internal consistency reliability and sensitivity, specificity, and diagnostic value of the MoCA test between groups respectively.

Results: The results showed a cut-off point of 26/27 as the probable point of cognitive impairment in mTBI. Also, in order to identify cognitive impairment in mTBI patients, this test reported sensitivity of 0.62 and specificity of 0.81 with Youden's index of 0.43.

Conclusion: In screening for possible mild cognitive impairment in mTBI patients, the MoCA is relatively useful and should not be used only as a substitute for a complete neuropsychological assessment with diagnostic purposes.

Keywords: Sensitivity, Specificity, Mild Traumatic Brain Injury, Montreal Cognitive Assessment.

Introduction

Traumatic brain injury consequences can cause a variety of pathological problems, including local dysfunction or disruption of neural networks and the cognitive status of patients.[1] As a result, researchers have focused on the cognitive sequelae of TBI over the last two decades.[1,2] Considering the current American Mild Traumatic Brain Injury Committee (AMTBI) diagnostic protocols,[3] patients with mTBI make up the majority of the TBI population, but only about 5% of them exhibit relevant or suspicious pathological findings.[4] Because of the inadequacy of existing diagnostic methods in identifying subtle changes in the structure and function of the brain,[5] the low cognitive performance in a large number of such patients is attributed to problems that existed before the injury, and, as a result, they do not receive medical attention.[6]

Clinical assessment of cognitive deficits will be difficult, especially when gross abnormalities indicating cognitive or functional deficits are not detected using diagnostic imaging tools.[7] As a result, an emphasis on the importance of understanding the subtle differences caused by mTBI[8] highlights the need for cognitive screening tools that will lead to optimal medical decision-making and facilitate individual and targeted therapeutic intervention.[9]

Cognitive screening is the starting point that used to identify cognitive disorders, dementia, and other neuropsychiatric syndromes. Professionals use a small number of short cognitive screening tools, and their effectiveness in some cases is unknown.[10] A comprehensive neuropsychological assessment for patients with disabilities and the use of a broad instrument in neuropsychological assessments, on the other hand, require time and money, as well as trained personnel. As a result, the existing hospital structure makes it impractical to provide the necessary facilities for their implementation in general.[11] This situation necessitated the use of cognitive screening tools, such as Mini-Mental State Examination (MMSE)[12] or Montreal Cognitive Assessment (MoCA)[13] to assess these patients' cognitive status in clinical examinations and tests.

Overcoming several limitations of other cognitive screening tools,[14] the MoCA test has been widely used as one of the most common tools in clinics and the first option among short comprehensive tools to assess cognitive disorders worldwide in more than 56 languages and dialects.[15] MoCA assesses more cognitive domains[16,17] than the MMSE, including short-term memory, spatial skills, executive function, attention, concentration, working memory, language, abstraction, and orientation.[18]

Many clinical tests are used to make enough assurance that ruling out or confirming the presence of a disease or to proceed with the diagnosis process. Under ideal circumstances, such tests would correctly identify all patients with the disease (sensitivity) and all healthy individuals (specificity).[19] The sensitivity and specificity of the MoCA test were reported to be 90-96% and 78-95%, respectively, using different cutoff points with scores of 23-26 as the diagnostic threshold for cognitive disorders.[11,20-23] Furthermore, while the MoCA test is one of the most recent and widely used tools for assessing functional cognitive changes and determining the number of cognitive abilities and impairments to predict functional outcomes in TBI patients,[2] the interpretation of the MoCA test results in clinical and health care settings is hampered by a lack of agreement on a cut-off score that accurately indicates cognitive impairment.[24]

Although some findings corroborate the usefulness of the MoCA test in assessing cognitive deficits of patients with mild TBI, the evidence in this field is still limited. There is no conclusive study has been found on the clinical effectiveness of the MoCA test in diagnosing cognitive impairment in mTBI patients. It is hence necessary to validate the MoCA test in comparison with other cognitive screening and assessment tools.[14] The question here is whether this tool can be used to assess patients with mild TBI and provide a specific cut-off point to distinguish patients with cognitive impairment.

Objectives

This study hence aims to determine the sensitivity and specificity of the MoCA test as a tool for rapid assessment of cognitive status in hospitalized patients to diagnose cognitive impairment in a sample of mTBI patients.

Methods

This was a cross-sectional, analytical observational study in which the study population consisted of all mTBI patients injured, were hospitalization at least 24h in the hospital, in Rasht (northern Iran) in the first half of 2022 and the participants were selected through sequential sampling. The G*Power software program (version 3.1.9.6) was used to calculate the sample size for estimating the sensitivity and specificity of the MMSE test according to the F test family.[25] Since the internal mechanism of discriminant analysis and one-way MANOVA (general effects test) are identical,[25] the sample size was obtained equal to 158. Of whom, 79 patients diagnosed with mTBI who were admitted to the trauma and neurosurgery ward, the neurological diseases ward, or the intensive care unit of Poursina Hospital of Rasht, visited the same hospital or Velayat Specialized Clinic for rehabilitation or other medical procedure and met the inclusion criteria were selected to be compared with 79 normal.

The inclusion criteria were being 18 to 65 years old, a Glasgow Coma Scale (GCS) score of 13-15, memory loss for events immediately before or after the accident less than 24 hours, loss of consciousness (LOC) less than 30 minutes, and any change in the mental state following the accident (stupor, confusion and …). The exclusion criteria were any serious mental and neurological disorder before TBI (e.g. schizophrenia, dementia, epilepsy, and Parkinson's based on clinical interviews with the patient and their support persons), a manifestation of symptoms later than 48 hours after the initial trauma (according to medical records), multiple and other major traumas (e.g. broken knee, chest, ruptured spleen), inability to respond, and unwillingness to participate in the study for any reason.

The participants and their families/concomitants were briefed on the research objectives and procedures. They were also assured that their information would be kept confidential and their withdrawal would not affect their treatment course. It is noteworthy that demographic data

were gathered only after obtaining the consent of the participants or their families. Then, the participants completed the Mini-Mental State Examination in an interview. This test was performed on 20 mTBI patients with an average time interval of 3 days to assess the retest reliability of the MoCA and the Pearson correlation coefficient was calculated between those two groups of scores. Participants also took the MMSE, in addition to the MoCA test, to determine the tool's concurrent and convergent validity. Pearson's correlation coefficient between these two tests was calculated for a group of 20 people.

Research Tools

Mini-Mental State Examination (MMSE)

It is a pencil-and-paper questionnaire that assesses five domains: orientation and spatial abilities, processing speed, attention and calculation, recall, and verbal memory in 5 to 10 minutes with the total score of 30.[26] According to Cronbach's alpha, the reliability of this test in patients with traumatic brain injury was reported to be 0.74.[27] Furthermore, Seyedian et al. demonstrated that the MMSE can distinguish the cognitive performance of two groups of dementia patients and normal people at a confidence level of 95%, and reported its internal reliability based on Cronbach's alpha coefficient of 0.81.[28]

Montreal Cognitive Assessment (MoCA)

This pencil-and-paper screening tool assesses seven cognitive domains: executive function, spatial ability, attention and concentration, memory, language, abstraction, and orientation[29] in less than 10 minutes with the total score of 30.[13] Furthermore, the cut-off point for the possible diagnosis of cognitive disorders is 26, which is used as the cognitive disorders diagnostic threshold.[30] People with 12 years of education or less are given an extra point to correct their scores on this test, up to a maximum score of 30.[31] The Cronbach's alpha and internal consistency of this test have been reported to be 96% and 82%, respectively.[32]

Statistical analysis

The continuous variables were expressed as the mean ± SD, and the categorical variables were presented as a percentage and frequency. All statistical analyses were performed with SPSS (version 16.0, SPSS Inc, Chicago, IL, USA). A "P-value" less than 0.05 was considered significant.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. The proposal for this project was approved with IR.GUILAN.REC.1400.038 code by the Ethics Committee of in Biomedical Research Center of Guilan University. All participants signed an informed consent form.

Results

Out of the 158 participants, 79 subjects were mTBI patients and 79 were normal individuals. Table 1 presents the demographics of the participants in both groups. The number of male ($p=0.016$) and single participants ($p<0.001$) was significantly higher in the mTBI group, and the academic level was also higher in the mTBI group ($p=0.027$). There was no significant difference between the two groups in other demographic properties ($p>0.05$).

Table 2 provides a summary of the percentage and frequency of background, clinical, and neuroimaging data of mTBI patients.

Figure 1 shows the distribution of MoCA scores between two groups of patients with mTBI and control group. The independent t-test results [Figure 1] indicate that the MoCA scores are lower in the mTBI group ($t=7.25$, $df=156$, $P<0.0001$). Therefore, the cognitive performance of the mTBI group was poorer than that of the normal individuals (mean 24.46 vs 28.37).

The test-retest reliability of the MoCA total score on 20 homogeneous mTBI patients with a mean interval of 3 days was $r = 0.89$ ($p<0.0001$). The MoCA scores were then correlated with MMSE scores to determine the convergent validity of the MOCA test. The findings revealed a strong, significant, and positive correlation between the scores of these two tests ($r=0.827$, $p<0.0001$, $n=20$). The results of the discriminant analysis to determine the sensitivity, specificity, and diagnostic value of the MoCA test to distinguish between mTBI patients and normal individuals in terms of cognitive status according to the cross-validated classification are shown in Table 3. The Youden index (or Youden's J statistic) is defined as $J=\text{sensitivity}+\text{specificity}-1$; was used to measure of overall diagnostic value.[33] If the Youden index is over 50%, then the test does meet empirical benchmarks for being administered for diagnostic purposes.

Table 1. Demographic characteristics of two groups of patients with mTBI (n=79) and control group (n=79)

Variables	mTBI (N=79)	Non-TBI (N=79)	Statistic	df	P value
Age	38.79-1.63	40.96-1.57	0.955	156	0.341
Sex, n (%)					
Females	7 (8.9)	18 (22.8)	0.139	156	0.016
Males	72 (91.1)	61 (77.2)			
Marriage, n (%)					
Single	33 (41.8)	17 (21.5)	3.30	156	0.001
Married	45 (57.0)	56 (70.9)			
Divorced	1 (1.3)	3 (3.8)			
Death of spouse	0 (0)	3 (3.8)			
Education, n (%)					
Low Literacy	4 (5.1)	1 (1.3)			
Elementary	11 (13.9)	10 (12.7)			
Middle school	26 (32.9)	20 (25.3)	2.238	156	0.027
High school	28 (35.4)	28 (35.4)			
University	10 (12.7)	20 (25.3)			
Job, n (%)					
Unemployed	9 (11.4)	5 (6.3)			
Worker	2 (2.5)	6 (7.6)			
Driver, Servicer, Housewife, Farmer, Student	39 (49.4)	35 (44.3)	1.481	156	0.141
Craftsman, Repairman, Foreman, University student	23 (29.1)	18 (22.8)			
Manager, Clerk, Employee, Seller	5 (6.3)	10 (12.7)			
Professional, Proficiency	1 (1.3)	5 (6.3)			

Table 2. Demographic and clinical characteristics of patients with mBTI

Value		N	%
Job (after trauma)	Unemployed	11	13.9
	Worker	2	2.5
	Driver, Servicer, Housewife, Farmer, Student	38	48.1
	Craftsman, Repairman, Foreman, University student	22	27.8
	Manager, Clerk, Employee, Seller	5	6.3
	Professional, Proficiency	1	1.3
Cause of Trauma	Car Accident	18	22.8
	Motorcycle Accident	27	34.2
	Pedestrian Accident	5	6.3
	Hit Objects	2	2.5
	Falling Down	3	3.8
	Conflict	8	10.1
	Drop Off	16	20.3
Brain fracture	None	61	77.2
	Linear	14	17.7
	Depressed fracture	3	3.8
	Basal fracture	1	1.3
Brain injury direction	None	23	29.1
	Right hemisphere	22	27.8
	Left hemisphere	27	34.2
	Bilateral injury	7	8.9
	None	15	19.0

Damaged area of brain	Forehead lobe	25	31.6
	Temporal lobe	18	22.8
	Parietal lobe	8	10.1
	Combination	13	16.5
Type of trauma	Focal	79	100
	Diffuse	0	0
Type of focal trauma	None	7	8.9
	Cerebral contusion	16	20.3
	EDH	19	24.1
	SDH	6	7.6
	SAH	7	8.9
	ICH	3	3.8
	Combination	21	26.6

Table 3. Sensitivity and specificity of MoCA (n=158)

Observed Membership	Predicted Membership		Sensitivity	Specificity
	TBI	**Non-TBI**		
mTBI	49	30	0.62	0.81
Non-TBI	15	64		
Overall diagnostic value (Youden's index)	0.43			

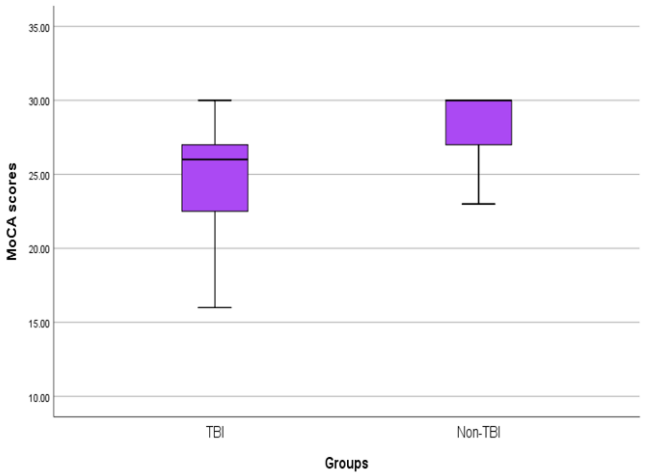

Figure 1. Comparison of the mean of MoCA total scores between the two groups of patients with mTBI and control group in two times of the test

Out of the 79 members of the mTBI group, 49 and 15 participants were identified as true patients and false normals, respectively. Therefore, based on Table 3, MoCA sensitivity was calculated to be 0.62. Furthermore, since 30 false patients and 64 true normal individuals were identified among the normal individuals, the specificity of the MoCA test was 0.81. In addition, the overall diagnostic value of the MoCA test (based on Youden index) was calculated to be 0.43, indicating that the MOCA test is not sufficiently reliable for diagnostic purposes.

The canonical discriminant function coefficients (0.306) were calculated with a constant value of -8.080 to determine the cut-off point of the MoCA test. The canonical structure, also known as canonical loading or discriminant loadings, represent correlations between observed variables and the unobserved discriminant functions (dimensions) [34 p.837]. The centroid of the group functions was -0.598 for the mTBI group and 0.598 for the normal group. In other words, the cut-off point of the test scores for both groups was 0.598, and the prediction of the participants' allocation is as follows based on the results of the regression equation:

Y= 0.306 (MoCA total score)- 8.080

If the total MoCA score is included in the above equation and the result is a negative number, the participant is predicted to be in the mTBI group. If the result is a positive number, the participant is predicted to be in the normal group. According to the findings, the mean MoCA score was 0.598 in the mTBI group and 0.598 in the normal group. As a result, considering the equal sample size of the two groups, the cut-off point for critical scores was calculated zero. Accordingly, the total MoCA cut-off point is calculated as follows:

Discriminant Score= 8.80 ÷ 0.306 = 26.40

DS= 26/27

Therefore, a MoCA test score of 26/27 or lower indicates that the mTBI patients have probable cognitive impairment and can be distinguished from normal people. Cronbach's alpha for determining the internal consistency reliability of MoCA in mTBI patients was 0.81 (n=79). The same reliability coefficient of the MMSE test was calculated to be 0.62 (n=79), indicating that the internal consistency of MoCA is more satisfactory. Table 4 compares the mTBI group and the normal group in terms

of the scores of subscales of the MoCA test.

When the mean scores in all MoCA subscales were compared, the significance level was equal to 0.0001 (smaller than 0.05). Thus, based on the mean values in Table 4, it can be concluded that the normal group's scores on all MoCA subscales were significantly higher than those of the mTBI group, and the normal group outperformed the mTBI group in all MoCA cognitive dimensions. Figure 2 shows the difference between the mTBI group and the normal group in terms of MoCA subscales.

Table 4. Comparison of the average of total scores and MoCA subscales between the two groups of patients with mTBI (N=79) and control group (N=79)

Subscale		Mean	SD	t	*df*	P-Value
Visual-spatial	mTBI	4.10	1.10	3.792	156	0.0001
	Non-TBI	4.65	0.69			
Naming	mTBI	2.12	0.40	6.326	156	0.0001
	Non-TBI	2.59	0.51			
Attention	mTBI	4.72	1.15	4.938	156	0.0001
	Non-TBI	5.50	0.81			
Language	mTBI	2.22	0.61	7.049	156	0.0001
	Non-TBI	2.81	0.39			
Abstraction	mTBI	1.06	0.62	4.749	156	0.0001
	Non-TBI	1.59	0.71			
Short-term memory	mTBI	4.08	0.96	7.187	156	0.0001
	Non-TBI	4.91	0.32			
Orientation	mTBI	5.56	0.90	3.728	156	0.0001
	Non-TBI	5.59	0.25			

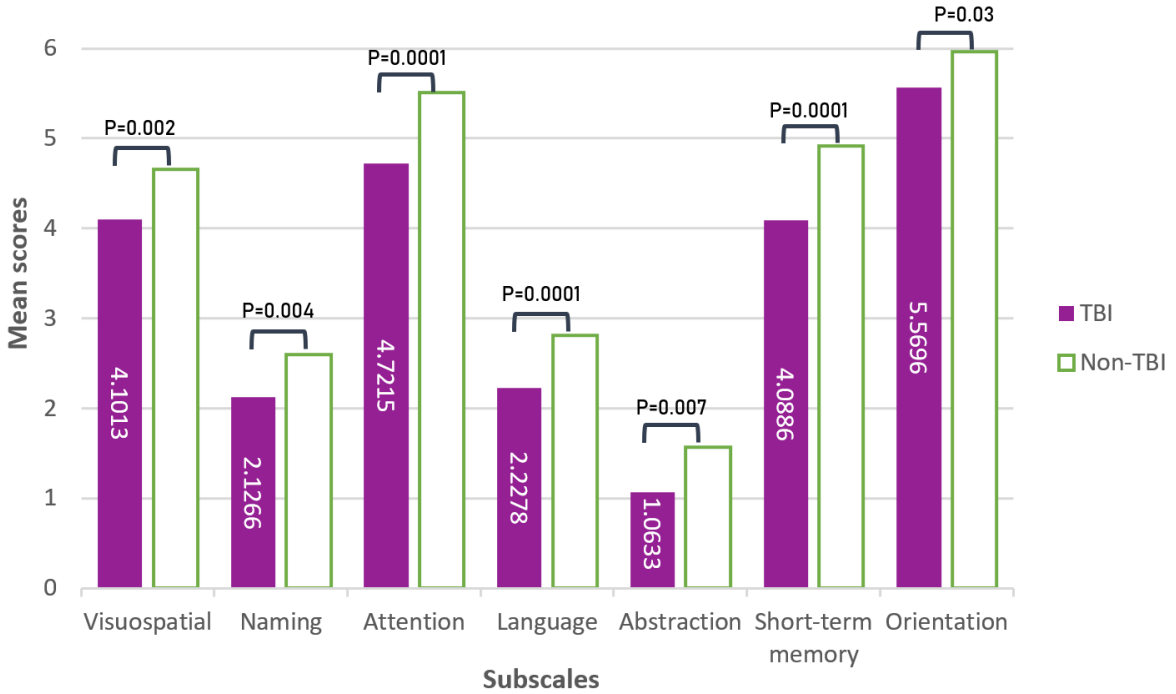

Figure 2. Comparison of MoCA subscales score between the two groups of patients with mTBI (N=79) and control group (N=79)

Discussion

Although more patients with TBI can survive because of clinical treatment improvement, their residual functional disorders have a serious effect on their prognosis and functional independence, putting a heavy burden on patients and their families.[35] Patients with mTBI performed worse in all cognitive components, including visual-spatial, naming, attention, language, abstraction, and orientation in the MoCA test, when compared to the control group. The study findings are consistent with

previous studies showing significant cognitive deficits in mTBI patients, primarily in episodic memory.[36-38]

It has already been known that memory impairment is one of the most common cognitive impairments following TBI, and it is the first significantly impaired function, as well as one of the last functions to return during the recovery process.[37] As a result, mTBI patients are expected to have poor performance in attention, memory, language, and executive functions. Frenette et al. also found a significant difference in the visual, spatial, and language components between normal people and mTBI patients and supported the findings of this study by presenting evidence of impairment in several cognitive areas in mTBI patients.[38]

Cognitive disorders are among the major causes of TBI-related disabilities and it is an important factor that has a detrimental effect on rehabilitation outcomes.[39] Therefore, findings proposed a cut-off point of 26/27 to distinguish mTBI patients with probable cognitive impairment from healthy participants. This is consistent with the findings of previous studies reporting a cut-off point of 26 or higher for the MoCA test in cognitive impairment screening.[13,36,39] On the other hand, although previous studies have acknowledged the optimal cut-off score of 26 and 27 for the mild cognitive impairment population[40] and patients with scores of 27 and above are considered normal, many of them may have significant cognitive deficits when compared to the normal population.

To explain this finding, if the patient has already achieved good functional results during partial recovery and is not significantly impaired in daily activities, subtle levels of cognitive impairment may not necessitate extensive neurological testing. It has also been discovered that individuals who score close to the proposed cut-off point are at a higher risk of receiving false negatives from academic remediation.[41] In such cases, clinical judgment about premorbid functioning (for example, occupational status) should be factored into total score interpretation, and cut-off point scores should be interpreted cautiously. In other words, misdiagnosis of cognitive disorders in healthy individuals causes anxiety for the patients and their families, and even being labelled.[40]

Based on the sensitivity and specificity results of this study, it is preferable that avoid the MoCA test as a substitute for comprehensive neuropsychological evaluation and diagnostic purposes. According to the results, the sensitivity and specificity of the MoCA test in the study sample were equal to 62% and 81%, respectively. This is consistent with the findings of some previous studies.[40-43] For example, Cheng et al. estimated the sensitivity and specificity of correct diagnosis of cognitive disorders and the absence of cognitive disorders by the MoCA test in the population of TBI patients to be 54% and 100%, respectively.[42] Gagnon et al. also reported that the MoCA test has a 69% sensitivity to identify cognitive disorders in people with mild dementia and the normal group and a specificity of 92% to correctly diagnose the absence of cognitive impairment.[43] Zhang et al., on the other hand, determined the MoCA test's sensitivity to correctly identify people with cognitive disorders and its specificity in people with mTBI were 66% and 87%, respectively.[44]

Several design features of MoCA can be mentioned in explaining the above findings, which likely explain its sensitivity in diagnosing mild cognitive disorders. When compared to MMSE, the MoCA memory test contains more words, fewer learning trails, and a longer delay before the recall. Executive functions, higher-level language abilities, and complex visuospatial processing can also be used to assess patients with mild cognitive impairment. In other words, the MoCA test assesses more cognitive domains, including various frontal lobe function assessment components (cube copying, letter fluency, letter tapping, clock drawing, and number sequencing).[17] This test also evaluates and measures more difficult things, such as executive functioning ability[44] and visuospatial abilities, which may be affected in the early stages of cognitive disorders.[45] As a result, it has been concluded that the MoCA test has a higher sensitivity in distinguishing the early stages of cognitive impairment.[46] Furthermore, the MoCA test can be used in secondary screenings because it assesses the most cognitive domains of any conventional cognitive screening tool.[47]

The study findings showed that the specificity of the MoCA test was higher than its sensitivity. This result can be explained by correcting academic grades and its effect on distinguishing normal people from people with cognitive disorders in the MoCA test because it seems that patients with a higher level of educational attainment are more familiar with the tool's tasks. In other words, it's important to note that individuals with abundant pre-disease cognitive reserves and higher education levels are less likely to experience cognitive deficits after a TBI. Given that low educational attainment is known as a moderating factor (or risk factor) in cognitive disorders, a point was added for patients with years of education less than 12 to control the said factor, according to MoCA instructions. Since patients who visit clinics frequently express memory complaints and are sometimes referred

from primary health care centers, a comprehensive neuropsychological assessment provides more insight into the general as well as domain-specific cognitive function deficits of such patients.[48] The study findings revealed the high specificity and low sensitivity of the MoCA test in identifying mTBI patients with a possible cognitive role. Therefore, this test is not recommended for identifying cognitive deficits, particularly in diseases with low prevalence, where tests with high sensitivity and low specificity (to avoid spurious healthy diagnoses even in spurious disease cases) are highly desirable.

The effects of cultural differences caused by place of residence and educational attainment on the MoCA performance were not investigated in this study. Particularly, educational attainment in the normal group was higher than that of mTBI patients, which would have confounding effects on the MoCA performance. On the other hand, attributing an extra score for years of education less than 12 may increase the rate of false negatives and misdiagnosis of cognitive impairment for people with cognitive impairment. As a result, it is suggested that MoCA scores be interpreted and considered when making diagnostic classifications, both "with" and "without" correction for education level and clinical intuition about prognostic function. With respect to educational attainment, Nasreddine et al.,[13] found that years of education affect MoCA performance. As a result, they suggested to add one 'correction point' to scores of individuals with 12 years of education or less. The sensitivity and specificity values obtained for the MoCA test in this study are far from ideal diagnostic values (90%),[49] which can result in a significant classification error that should be considered in clinical decision-making. Another limitation of this study was the absence of different groups in terms of TBI severity (mild/moderate/severe). Therefore, future studies are recommended to investigate the entire spectrum of traumatic brain injury in larger samples. Finally, there was no gold standard for diagnosing cognitive impairment (*The Diagnostic and Statistical Manual of Mental Disorder DSM-5-TR*) using the receiver operating characteristic curve (ROC curve) in this study.

Conclusions

MoCA is a screening tool that addresses a few aspects of clinical criteria for the diagnosis of cognitive disorders and cannot be considered an alternative to comprehensive neuropsychological tests during the follow-up and initial cognitive treatment process. A score of 26/27 or lower may indicate mTBI patients with probable cognitive impairment. Furthermore, since the incidence of cognitive deficits varies in the mTBI population, it is critical to understand cognitive and functioning impairment in this population. This highlights the role of predictive tools in primary care and degenerative disease prevention. Doctors can improve their intervention strategies for improving their patient's quality of life and delay the progression of cognitive disorders by using a brief cognitive screening tool such as MoCA. Such tools help them eliminate the need for a wider neuro-psychological evaluation, speed up the clinical evaluation process, and provide mTBI patients with faster and less expensive hospital services.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

mild traumatic brain injury: mTBI;
Montreal Cognitive Assessment: MoCA;
Mini-Mental State Examination: MMSE;
American Mild Traumatic Brain Injury Committee: AMTBI;
Glasgow Coma Scale: GCS;
loss of consciousness: LOC.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

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Availability of data and materials

The data used in this study are available from the corresponding author on request.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The proposal for this project was approved with IR.GUILAN.REC.1400.038 code by the Ethics Committee of in Biomedical Research Center of Guilan University. All participants signed an informed consent form.

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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