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ORIGINAL ARTICLE

Serum Malondialdehyde As A Predictor Of Post-Stroke Cognitive Impairment In Ischemic Stroke Patients

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ABSTRACT

Background: Cognitive impairment is a common consequence of stroke and is associated with poor functional outcome and higher risk of recurrent cerebrovascular stroke. Serum Malondialdehyde (MDA) is used as a biomarker to measure the oxidative stress. The association of serum MDA levels in acute ischemic stroke and post-stroke cognitive impairment (PSCI) has infrequently been studied. This study aimed to identify the relationship between serum MDA levels in the first week after acute ischemic stroke, and PSCI after three months.

Patients and methods: Thirty-seven patients 17 males (45.9%) and 20 females (54.1%) with acute ischemic stroke recruited from Neurology critical care units, Zagazig University hospitals were prospectively enrolled in follow-up cohort study. Serum MDA was measured within 24 h after admission. Cognitive outcome after three months was assessed by Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA).

Results: 43.2% of patients had no cognitive impairment while 35.1%had mild and 21.6% had severe cognitive impairment according to MMSE and 59.5%had unfavorable MoCA outcomes. There was a significant negative correlation between serum MDA and MMSE and MoCA.

Conclusion: Increasing MDA serum level had a significant effect on development of PSCI.

Keywords: Ischemic Stroke, Malondialdehyde, Cognition.

1. INTRODUCTION

Stroke is one of the foremost causes of worldwide death. One-third of these cases are fatal, and survivors usually have extended or permanent disabilities [1]. The ischemic injury leads to interruption of blood brain barrier, inflammation, and oxidative stress. Protein, lipids, and DNA are disrupted by reactive oxygen species [2]. Malondialdehyde (MDA) is the end-product of lipid peroxidation. This compound is one of the numerous reactive electrophile species that cause cellular toxicity. This aldehyde is usefully used as a biomarker to quantity the oxidative stress level [3].Cognitive impairment is a progressively predominant consequence after stroke and its occurrence has been linked with poor functional outcome and increasing the risk of recurrent cerebrovascular stroke even in patients with fruitful clinical recovery [4,5]. Suggestions have established the vigorous role of oxidative stress pathways in the directive of cognitive impairment associated processes, including neurodegeneration, vascular inflammation, and blood–brain barrier disruption, entirely of which show the possible relationship between oxidative stress and cognitive impairment [6,7].The present study was conducted to determine whether there was an association between the serum MDA level and short-term cognitive outcome of acute ischemic stroke.

2. THE PATIENTS AND METHODS

The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.We prospectively analyzed 37 consecutive patients 17 males (45.9%) and 20 females (54.1%) with first-ever ischemic stroke admitted within 7 days of the stroke symptoms onset to the Neurology Critical Care and Stroke Units, Neurology Department, Zagazig University Hospitals between May 2018 and March 2019. Patients were diagnosed according to the World Health Organization (WHO) criteria [8] and confirmed by brain computed tomography (CT) and / or magnetic resonance imaging (MRI).

Our exclusion criteria were: Hemorrhagic stroke, Patients with concomitant Alzheimer's disease or other neurological diseases that possibly affect cognition (e.g., Parkinson's disease, multiple sclerosis), Patients with severe medical illness (e.g., terminal cancer, hepatic or renal failure,

chronic inflammatory, autoimmune or hematologic diseases, thyroid dysfunction), History of depression (clinical diagnosis or previous treatment) or other psychiatric disorders, Patients with residual aphasia and dysarthria or hearing impairment, History of Drugs that can disturb cognitive functions e.g., anti-Parkinson, antiepileptic, antipsychotic and alcohol. Patients who died during follow up were also excluded from the study.All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national). Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University approved the study protocol (No. 4815). An informed consent was obtained from all participants, or their first-degree relatives and they were told about the aim of the study, and were informed that the data would be used for scientific purposes only. On admission: all patients in this cohort study were subjected to the following: Clinical assessment: including detailed history taking, full examination (general and neurological), and laboratory investigations including Complete Blood Count, blood glucose level, liver, kidney and thyroid functions, lipid profile. Also, the patients in the study had CT scan and/or MRI of the brain done to verify the diagnosis. Serum MDA level was done for all stroke patients after admission within 24h at Central Research Lab, Medical Biochemistry and Molecular Biology Department, Faculty of Medicine Zagazig university. Serum malondialdehyde (MDA) level, as Oxidative stress marker, measured according to Ohkawa et al. [9], The principle of the method is based on that MDA can react with Thiobarbituric Acid in acidic media at temperature of 95^{o} C for 30 min. to form Thiobarbituric Acid reactive product. The resultant pink product can be measured spectrophotometrically at 534 nm.

Calculation: Malondialdehyde in serum = (A Sample ÷ A Standard) x 10 nmol/ L.

The serum concentration of MDA was expressed in nmol/mL. The calibration curve was organized with authentic MDA standards ranging from 0–20 nmol/mL. The detection limit of this assay was 0.079 nmol/mL; the intra- and intraassay coefficient of variability were 1.82% and 4.01%, respectively. To avoid the possible dispersion of serum MDA level results, all the samples were handled at the same time, at the end of the recruitment process. MDA determination was performed by a laboratory technician blinded to all clinical data.Follow-up assessment of the patients were done three months later by using Mini-Mental State Examination (MMSE) for assessing the cognition three months post stroke. Scores below 24 were considered to be revealing of cognitive impairment [10], in this study a score of (24-30) is measured no impairment, (18-24) is measured mild impairment and (0-17) is measured severe impairment. Also, we use Montreal Cognitive Assessment (MoCA) for assessing the degree of cognitive impairment. The MoCA is a screening test (one-page 30-point) administered in about 10 min and was planned as a rapid screening instrument for mild cognitive impairment (MCI). It evaluates different cognitive domains: attention and concentration, language, memory, executive functions, visuo-constructional skills, conceptual thinking, calculations and orientation. The total conceivable score is 30 points; a score of 26 or above is measured favorable outcome; and a score below 26 considered unfavorable outcome and an extra point is added if the person has 12 years or less of formal education [11,12].

In addition, using of Hamilton Depression Rating Scale (HAM-D) for evaluating and excluding the post stroke depression [13].

3. STATISTICAL ANALYSIS

The data were tabulated and statistically analyzed using Statistical Package for Social Science (SPSS) Program version 14.0.0 software package. The qualitative data were presented in the form of numbers and percentage. The T test and one-way ANOVA were used as tests of significance. Receiver operating characteristic analysis was performed to estimate the area under the curve (AUC), and we used the likelihood ratio between sensitivity/1-specificity as criteria to select the cutoff of the serum MDA level to discriminate the presence of cognitive impairment. Pearson's Correlation co-efficient rank test; it was used to rank different variables against each other in linear correlation which may be positive or negative. Probability (P value of > 0.05 indicates non-significant results; P value < 0.05 means significant difference, p> 0.001 for highly significant result).

4. RESULTS

This cohort study was conducted on 37 first ever acute ischemic stroke patients (17 males and 20 females), their ages ranged from 53 to 72 with a mean age (62.95 ± 4.21(SD)) (Table 1), who were admitted in Neurology critical care unit and stroke units of Zagazig University Hospitals within seven days of symptoms onset.The value of MDA biomarker in the 1st 24 h of admission in the studied ischemic stroke patients presented in (figure 1), we found that 42.2% of the studied ischemic stroke patients had MDA level more than 2.65 nmol/L compared to 57.8% had MDA level less than 2.65 nmol/L. Moreover, the ROC curve

analysis showed an area under curve (AUC) value of 0.751 (95% CI, 0.597 – 0.907; p < 0.05), and the optimal cut-off value for MDA as a diagnostic marker of stroke was 2.65nmol/L, which yielded a sensitivity of 66.7% and a specificity of 87.5% as shown in (figure 2) and (table 2).Regarding MMSE, 43.2% had no impairment, 35.1% had mild impairment and 21.6% had severe impairment. Furthermore, 59.5% had unfavorable MoCA outcome compared to 40.5% had favorable outcome as shown in (table 3).

The relation between serum MDA and post-stroke cognitive outcome measures are shown in (table 4). There was statistically significant increase in mean value of MDA in patients with mild (p<0.05) and severe (p<0.001) impairment compared to patients without impairment according to MMSE. However, there was not statistically significant (p> 0.05) increase in mean value of MDA in patients with severe impairment compared to patients with mild impairment. Also, there was statistically highly significant (p< 0.001) difference between patients with unfavorable and favorable outcomes of MoCA regarding mean values of MDA. Furthermore, there was statistically highly significant (p < 0.05) negative correlation between MDA and MMSE, and between MDA and MoCA as shown in (table 5), (figure 3) and (figure 4).

Table 1: Age and sex characteristics of the studied ischemic stroke patients**.**

Demographic data	**The studied patients(n=37)**	
	No.	**%**
Age (years):		
Mean ± SD	62.95±4.21	
Rang	53-72	
Sex:		
Male	17	45.9
Female	20	54.1

Table 2: Roc curve analysis of the MDA in discriminating the cognitive impairment post stroke**.**

Curve characteristics	**Values**
Area under the curve	0.751
Standard error	0.078
95% confidence interval	0.597 – 0.907
p-value	<0.05*
Predictive characteristics	
Best cut off value	2.65
Sensitivity	66.7%
Specificity	87.5%
PVP	87.5
PVN	66.7
Accuracy	75.7

Table 3: cognitive outcomes of patients after three month of stroke onset

Outcome		**No.**	**Percent (%)**
MMSE (n=37)	No impairment (24-30)	16	43.2
	Mild impairment (18-24)	13	35.1
	Severe impairment (0-17)	8	21.6
MOCA (n=37)	Unfavorable outcome (< 26)	22	59.5
	Favorable outcome (≥26)	15	40.5

Table 4: Relation between MMSE and MDA and MOCA and MDA in ischemic stroke patients

		MDA (Mean ±SD)	F	P -value
MMSE	**No impairment (n=16)**	2.23±1.6	6.97	0.003**
	Mild impairment (n=13)	3.96±2.2[a]		
	Severe impairment (n=8)	5.14±1.9[bc]		
		MDA (Mean ±SD)	**t**	**P- value**
MOCA	**Unfavorable outcome (n=22)**	4.29±2.14	3.291	0.001**
	Favorable outcome (n=15)	2.25±1.63		

****: highly significant (p<0.01) [a]p<0.05,**
[b]p<0.001 compared to no impairment score
[c]p>0.05 compared to mild impairment score

Table 5: Correlation between MMSE, MOCA and Malondialdehyde

Variable	MDA	
	R	**P-value**
MMSE	-0.59	**<0.001****
MOCA	-0.49	**0.001****

r: coefficient of spearman's correlation **: significant (p< 0.05)

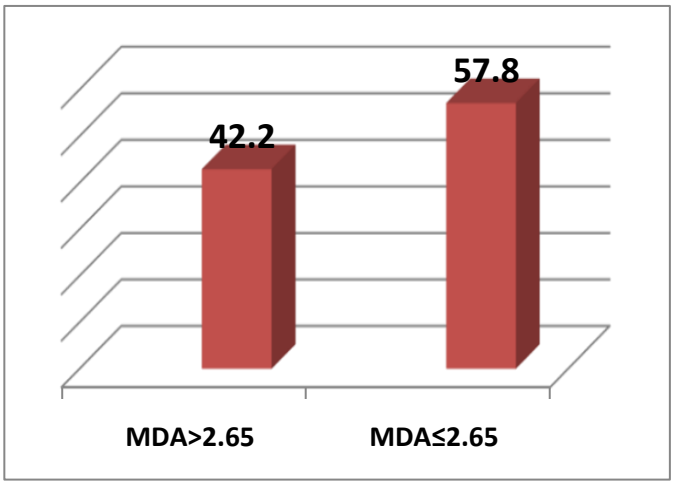

Figure1. Malondialdehyde distribution in the studied ischemic stroke patients

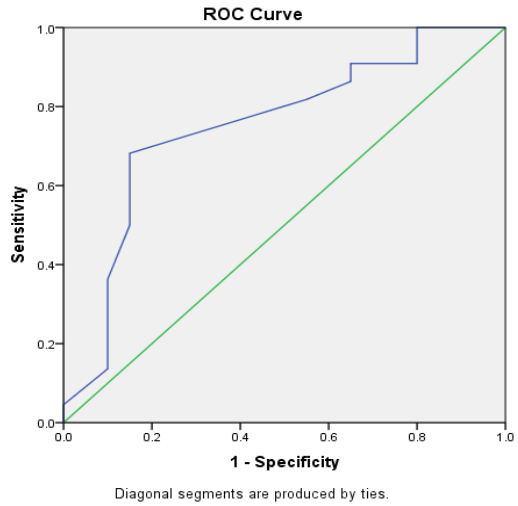

Figure2. Roc curve analysis of the MDA in discriminating the cognitive impairment post stroke

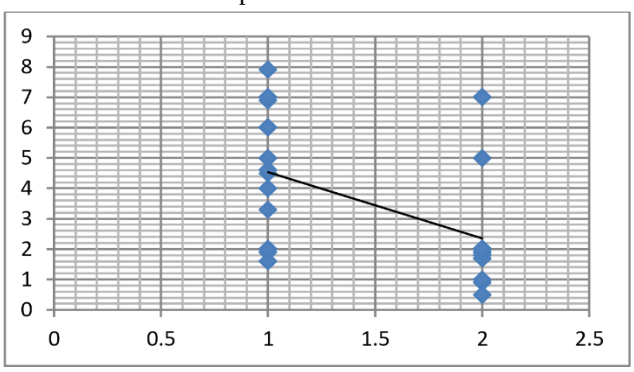

Figure 3. Correlation between MDA and MMSE

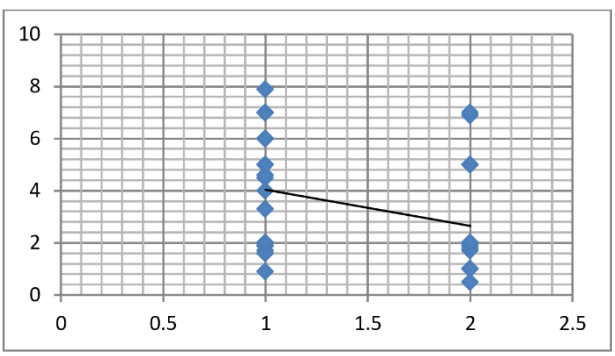

Figure 4. Correlation between MDA and MOCA

5. DISCUSSION

Cognitive impairment is a communal consequence of stroke and one of the major determinants of poor outcome [14]. Even minor cognitive discrepancies resulting from stroke can distress patients' quality of life, independent functioning, and occupational capabilities [15]. Malondialdehyde (MDA) is a final-product of the radical-initiated oxidative breakdown of poly-unsaturated fatty acids and, consequently, it is a commonly measured as oxidative stress biomarker [16].

Cumulative suggestion has verified the dynamic role of oxidative stress pathways in the regulation of cognitive impairment related diseases, which specify the potential association between oxidative stress and cognitive impairment [6]. The importance of this study is to assess the serum level of MDA and its association with short-term cognitive outcome in acute ischemic stroke.

In the present study, we found 35.1 of patients had mild cognitive impairment (18-24) and severe impairment MMSE (0-17) was in 21.6%. Also, Unfavorable outcome MoCA (below 26) was in 59.5%.Our results regarding the cognitive impairment, it is close similar to the previous study done by Valkova et al. [17] who recorded that using MMSE (MMSE points below 24), was found in 24.07% of stroke survivors and with using MoCA, they obtained cognitive impairment (MoCA points below 26) in 64.81% of stroke survivors. Additionally, post stroke cognitive impairment results in our study were similar to that observed in previous studies; Sundar and Adwani [18] who found that 31.7% patients had impaired MMSE (score < 24) at three months post ischemic stroke cognitive impairment and Godefroy et al. [19] who reported that 67% of their patients with MMSE <23, were categorized as cognitively impaired and the frequency of impaired MoCA below 26 was 82%.In our study 42.2% of the studied ischemic stroke patients had MDA level more than 2.65 nmol/L, while 58.8% of patients had MDA level less than 2.65 nmol/L. Similar percentages of elevation of MDA level in acute ischemic stroke patients (21-52%) were recorded by D'Souza et al. [20], Premnath et al. [21], Tsai et al. [22], Yaseen et al. [23], Lorente et al. [24], Silina et al. [25] and Jena et al. [26].In this study ROC curve analysis was used to evaluate the usefulness of MDA to discriminate the presence of cognitive impairment and showed an area under curve (AUC) value of 0.751 (95% CI, 0.597 – 0.907; $p < 0.05$), and the optimal cut-off value for MDA as a diagnostic marker of stroke was 2.65nmol/L, which yielded a sensitivity of 66.7% and a specificity of 87.5%. This coped with study conducted by Liu et al. [27] who reported that The AUC value for MDA in discriminating the patients with cognitive impairment from the non-post stroke cognitive impairment group was 0.793 (95% CI, 0.731–0.856; $p < 0.001$). The optimal cut-off for MDA was 2.59 nmol/L. As concerning the relationship between the PSCI (assessed by MMSE and MoCA) and MDA mean values, there was statistically significant upsurge in mean value of MDA in patients with mild and severe impairment of MMSE in comparison to patients without impairment. Though, there was no statistically significant rise in mean value of MDA in patients with severe impairment in comparison to patients with mild impairment. Likewise, there was statistically exceedingly significant difference between patients with unfavorable and favorable outcomes of MoCA concerning mean values of MDA; we found statistically significant negative correlation between MDA level and MMSE and between MDA and MoCA.

Our patient's outcome as regards the cognitive impairment is quietly similar to the previous studies like that of Liu et al. [27] who reported that there was a significant negative correlation between MDA and MMSE scores in the stroke patients, and Torres et al. [28] who stated that MMSE was negatively associated with MDA levels, which suggests that lipid peroxidation is an early occasion in the development of dementia.

To the best of our knowledge none of the previous studies correlated MDA levels with post-stroke cognitive impairment using MoCA.

Increased MDA levels are largely considered as signs of triggered oxidative stress pathway. Oxidative stress may impact post-stroke cognitive impairment through diverse mechanisms. The interruption of the blood-brain barrier subsequent ischemia is alleged as an imperative initiating factor in dementia [29]. The occurrence of hypoxia-ischemia and vascular risk factors for vascular cognitive impairment are satisfactory to activate oxidative stress responses [30]. Numerous research specify that oxidative stress is implicated in the ischemic brain injury pathogenesis through cell death pathways [2]. The limitation of this study, that serum MDA levels were only assessed for all patients within the first 24 hours after admission. It may be indispensable to conduct an additional longitudinal study measuring serum MDA levels at multiple time points after stroke and assessing the predictive value of MDA at later times. The inconsistency of results is credited to an inadequate sample size study differences in population- and patient level characteristics (i.e., socio-demographics and clinical issues), methodology (i.e., the diagnostic criteria for PSCI), and the time interval between stroke and cognitive

assessment hypothetically explain inconsistent reports of differences in post stroke cognitive impairment (PSCI). Our findings should be considered preliminary, and additional clinical trials with MDA should be led to define whether oxidative stress modulation in stroke patients might support therapeutic indication in order to impact the PSCI. Consequently, upcoming studies will be compulsory to explore whether MDA imitates the cognitive impairment severity after stroke in a larger sample and in groups with more homogeneousness.

6. CONCLUSION

We concluded that high serum MDA levels at admission are associated with the development of post-stroke cognitive impairment after acute ischemic stroke.

Conflict of interest

Authors report no conflict of interest.

Financial Disclosure

Authors report no financial Disclosures

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