ORIGINAL ARTICLE

Role of microRNA-223 and High Mobility Group box 1 as Predictors of Drug-resistant Epilepsy

[1]Randa A. El Zohne, [1]Osama B. Seddik, [2]Wafaa M. A. Farghaly, [2]Mohamed M. Shehab, [1]Safaa A. Samir, [3]Menna S. Al-Adl*, [1]Yousra M. Mammdoh

[1]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt
[2]Department of Neurology and Psychiatry, Faculty of Medicine, Assiut University, Assiut, Egypt
[3]Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt

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Corresponding Author:
Menna S. Al-Adl, PhD
PhD of Biochemistry
Biochemistry Division, Chemistry Department, Faculty of Science, Mansoura University, Mansoura, Egypt
Postal code: 35511
Tel.: +201010149307
mennasalah1992@yahoo.com
0009-0000-3021-5748

ABSTRACT

Background: *Epilepsy is a prevalent neurological illness, impacting about 70 million individuals around the world. Although a large number of patients are controlled with anti-seizure drugs (ASDs), 30%–40% of patients fail to be controlled and develop drug-resistant epilepsy (DRE).* ***Objectives:*** *This study aimed to identify miRNa-223 expression and serum level of high mobility group box 1 (HMGB1) as biomarkers for detecting DRE.* ***Methodology:*** *This case-control study comprised 96 subjects categorized to three groups: group I: 46 patients with genetically presumed epilepsy who having DRE; group II: 25 patients with medically controlled genetically presumed epilepsy and group III: 25 healthy individuals. MiRNA-223 expression level was measured by real-time PCR and serum HMGB1 was estimated by ELISA technique.* ***Results****: MiRNA-223 expression serum HMGB1 and hs-CRP levels in epilepsy patients were considerably greater than in patients with controlled epilepsy and in healthy controls ($p<0.001$ for all). The predictive ability of miRNA, HMGB1 and hs-CRP for the detection of epilepsy and DRE using the ROC curve analysis revealed good sensitivities and specificities.* ***Conclusion:*** *MiRNA-223 expression and serum HMGB1 and hs-CRP levels are important biomarkers for diagnosis of epilepsy in suspicious cases. They are also significant for predicting DRE which may pave the way to the development of new antiepileptogenic drugs.*

INTRODUCTION

The neurological condition, epilepsy is typically defined as an imbalance between the brain's excitement and inhibition. The International League against Epilepsy estimates that more than 70 million individuals suffer from this condition globally[1]. Epilepsy is associated with physical, cognitive, psychological, and social impairments and is a main risk factor for unexpected death[2].

Research on epilepsy has historically produced important medication discoveries. Patients have benefited from the anti-seizure drugs (ASDs) by having fewer or no seizures. Regretfully, not all individuals with ASDs experience the ideal outcome of having seizures eliminated with little side effects[3]. Even after receiving appropriate treatment for multiple ASDs, many people still experience seizures; these individuals are referred to have drug-resistant epilepsy (DRE) [3].

The overexpression of multidrug transporters, such as multidrug resistance gene 1 (*MDR1*), which codes for a P-glycoprotein (p-gp), in brain astrocytes is an important mechanism of drug resistance in epilepsy. This mechanism stops a drug from building up in cells at a sufficient concentration. Evidence currently available indicates that *MDR1* expression is targeted and modulated by hypoxia-inducible factor 1α (HIF-1α). Research indicates that a diminished concentration of ASDs in the brain results from the coordinated overexpression of *MDR1* and *HIF-1α*[4].

Surgically removing the epileptic foci can help individuals with DRE who have a focal onset stop having seizures[5]. Neuromodulation techniques like vagal nerve stimulation and the ketogenic diet are additional methods for treating DRE that are better than continuously administering ASDs[6]. Thus, to choose the best clinical treatment and improve clinical response and patients' quality of life, it would be advantageous to find novel biomarkers that can predict the DRE early[7].

Evidence suggests that miRNAs may be used as biomarkers for epilepsy and other brain damage. Through controlled exoplasm release, damage, or even breakage of the blood-brain barrier (BBB), a pool of brain-expressed miRNAs may seep into the extracellular fluid and enter the bloodstream[8].

These miRNAs stay in circulation for some time after being released because they either encapsulate themselves in extracellular vesicles or form a stable combination with blood proteins. Therefore, epilepsy biomarkers are crucial because they may be used to

diagnose, determine the likelihood of developing DRE, and track treatment[8].

Alterations in miRNA expression and activity may have an impact on cellular processes, and miRNA can influence the synthesis and molecular structure of different proteins. In fact, oligonucleotide-mediated miRNA expression can readily result in noticeable alterations in gene expression. Because of these characteristics, miRNAs are valuable indicators for epilepsy and possible new targets for treatment[9].

The prognosis of epileptic patients and the selection of appropriate treatment may both be enhanced by the discovery of circulating biomarkers for refractory epilepsy[10]. Numerous investigaions have examined the potential role of miRNAs in drug resistance mechanisms via the HIF-1α pathway[11]. MiRNA-223 has been shown to affect inflammation in the central nervous system (CNS) and control microglial autophagy. Analysis of brain tissue samples taken from epileptic patients indicates that the onset and course of epilepsy are accompanied by a widespread disruption of gene expression[12].

The precise mechanisms behind pharmaco-resistance in temporal lobe epilepsy and how they are regulated remain unknown, despite the fact that it is well known that miRNA activity regulates a number of genes implicated in cell proliferation, apoptosis, neuroinflammation and autophagy[13].

The chromatin-binding protein HMGB1, which controls gene transcription, acts as a crucial molecular pattern molecule linked to damage, after being released[14]. There is compelling evidence that dysregulated neuroinflammation in the brain may influence how people react to ASDs, as well as contribute to ongoing seizures and the advancement of DRE. Sterile neuroinflammation triggered by epileptogenic damage and recurrent seizures is mediated by HMGB1. In human DRE foci, HMGB1 is upregulated in BBB neurons, glia, and endothelial cells[15].

The active release of HMGB1 by neurons and glial cells during inflammasome activation activate the Toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products on target cells[15]. The HMGB1/TLR4 axis has a crucial function in triggering neuroinflammation after brain trauma that results in epilepsy[16]. Additionally, patients who are resistant to ASDs have higher levels of both *HMGB1* and *TLR4* expression, which is related to a higher risk and severity of epilepsy[17].

The overexpression of P-gp, a BBB protein that is increased in DRE foci and extrudes different ASDs from the brain, is facilitated by the HMGB1-TLR4 axis. Therefore, HMGB1 may be useful as a mechanistic biomarker for medication resistance and may be linked to epilepsy, according to the body of evidence[18].

In order to guide appropriate care and pave the way for future pharmacological trials, this study sought to assess the roles of miRNA-223 and HMGB1, along with high sensitive CRP (hs-CRP) in the diagnosis of epilepsy and early prediction of DRE.

METHODOLOGY

Study population

This case-control study consists of 96 subjects categorized into 3 groups:

- **Group I**: 46 patients between 8-48 years with genetically presumed epilepsy who having DRE (failure to achieve sustained seizure freedom during the last 6 months) after adequate and well-tolerated trials of two ASDs. Patients' age ranged between 8-48 years.
- **Group II**: 25 patients with medically controlled genetically presumed epilepsy (who are seizure-free for at least the last 6 months).
- **Group III:** 25 healthy persons who are neurologically free, having no history or family history of epilepsy.

The samples were collected from the Outpatients of the Epilepsy Clinic and Inpatients of the Neurology and Psychiatry Department of Assiut University Hospital between December 2022 to November 2023. An informed consent was taken from all contributors according to the guidelines of the Assiut Faculty of Medicine Ethics Committee. Practical work was carried out at the Clinical Pathology Department, Assiut University Hospital. Clinical trials ID: (NCT05555537). The Exclusion criteria involved patients with symptomatic epilepsy (encephalitis, cerebrovascular, tumor, syndromic epilepsy, traumatic or febrile convulsion) and those with other CNS disorders, including Alzheimer's disease, Parkinson's disease, major depressive disorder, autism, or multiple sclerosis. Patients who are non-compliant with antiseizure medication and those with non-neurological disorders such as tumors, renal, hepatic, and cardiovascular diseases, were excluded from this study.

All groups underwent a thorough history taking with a focus on (age of onset, seizure semiology, antiseizure medications used, and compliance), in addition to a full clinical and neurological examination. For patients only**,** a video of electroencephalography (EEG) was performed, along with MRI of the Brain to exclude symptomatic epilepsy.

Sampling:

Four ml of venous blood were collected under aseptic conditions from all participants. The blood sample was divided into: 2 ml of blood for separation of serum by centrifuging for 5 min at 2000 rpm, for routine

HMGB1 assay and 2 ml of blood were placed into EDTA-coated tube for extraction of RNA. The extract was further kept at -80 °C till assay.

Methods:

Serum HMGB1assay

Serum HMGB1 was measured by the ELISA technique using a BioTek Plate Reader, USA analyzer; Human HMGB1 ELISA Kit (Cat. No. E-EL-H1554) according to the manufacturer's instructions.

MiRNA-223 expression assay

Extraction of miRNA-223 was done using miRNeasy Mini kit (QIAGEN, Germany, Cat. No. 217204). Complementary DNA (cDNA) was obtained using cDNA Reverse Transcription Kits (Thermo Fisher, Waltham, USA, Cat. No. 4374966). Then cDNA amplification and detection were done using BioTek Epoch microplate, USA, by SYBR Green PCR Master Mix (2X) (QuantiTect, Waltham, USA, Cat. No. K0221). All primers were synthesized by (Thermo Fisher Scientific, Waltham, USA), as displayed in **(Table 1)**[20] and all the methods were according to manufacturer protocol.

Table 1: Primers used in real time PCR of MiRNA

Sequence Name	Forward 5'-3'	Reverse 3`-5`
miRNA-223	AGCCGTGTCAGTTTGTCAAAT	GTGCAGGGTCCGAGGTC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

Each sample has 2 reaction tubes; one for the housekeeping primer (SNORD) tube 1 and one for miRNA-223 tube 2 primer assay. In each tube of the sample 12.5 µl of SYBR Green was mixed with 2.5 µl RNase- ree water and 5 µl of cDNA. Lastly, 2.5 µl of each SNORD (forward and reverse) primers were added to tube 1 and 2.5 µl of each (forward and reverse) miRNA-223 primer to tube 2, so, the final volume was adjusted to be 25 µl in each tube. The U6 acted as an internal control and the reaction mixture was dispensed into the proper place/ well in the 7500 Fast Real-Time PCR System after sealing with the appropriate provided caps. Delta-Delta method for comparing Relative Quantitation was applied for results interpretation[19]:

ΔCt Sample= $Ct_{miRNA223}$-Ct_{SNORD}

ΔCt Control= $Ct_{miRNA223}$-Ct_{SNORD}

Relative quantitation = $2^{-[^{\Delta Ct}\text{Sample} - {}^{\Delta Ct}\text{Control}]} = 2^{-\Delta\Delta Ct}$

Relative quantitation for control = 1, if ≥ 1 means positive expression and < 1 means low expression.

Statistical Analysis

All statistical computations were performed using SPSS Inc. (Chicago, IL, USA, version 22). Data were presented statistically using mean ± standard deviation (±SD), median and range for non-normal distribution, frequencies (number of cases), and relative frequencies (percentages) when appropriate.

The Pearson correlation test was applied to determine the relationship between several variables. Receiver Operating Characteristic Curve (ROC) analysis was utilized to determine the optimal cut-off values for validating the prediction of epileptic seizures and their resistance status using several plasma biomarkers. P-values are always two-tailed and significant at the 0.05 level.

RESULTS

Table 2 showed no significant variance between the 3 groups concerning age or sex distribution ($p>0.05$). The median disease duration in the DRE group was 9 years versus 10 years for the CE group, while the median duration since last fit was 5 days for the DRE group versus 720 days for the CE group ($p<0.001$). There is no significant change between the DRE and CE groups regarding the type of epilepsy. Most cases of both groups had generalized convulsions.

Table 3 showed that patients with DRE had significantly higher miRNA-223 expression level than patients with CE and healthy controls ($P<0.001$ for both) and patients with CE had significantly higher miRNA-223 expression level than controls ($p=0.013$).

Patients with DRE had considerably raised serum HMGB1 level compared to CE patients and healthy controls ($P<0.001$ for both), whereas no significant difference was observed in HMGB1 level between cases with CE versus healthy controls ($P=0.171$).

Moreover, patients with DRE had significantly higher hs-CRP levels compared to patients with CE and healthy controls ($P<0.001$ for both). At the same time, no substantial difference was detected between cases with controlled epilepsy versus healthy controls ($P=0.639$).

Table 2: Demographic data, duration and type of fits between both studied groups.

Variable name	DRE (n=46)	CE (N=25)	HC (N=25)	P value
Age (years)				
Mean ± SD	28.7 ± 9.20	30.5 ± 11.08	34.1 ± 7.8	0.071
Range	8 – 48	10 – 55	15 – 51	
Sex, n (%)				
Males	23 (50%)	12 (48%)	12 (48%)	0.981
Females	23 (50%)	13 (52%)	13 (52%)	
Disease duration (years)				
Median (range)	9 (1 – 30)	10 (3 – 36)	NA	0.823
Duration since the last fit (days)				
Median (range)	5 (1 – 170)	720 (180 – 1800)	NA	**<0.001*** **
Type of fit, n (%)				
Generalized convulsion	41(89.1%)	24 (96.0%)	NA	0.261
Psychomotor fits	2 (4.3%)	1 (4.0%)		
Atonic fits	1(2.2%)	0 (0.0%)		
Focal with impaired awareness	1(2.2%)	0 (0.0%)		
Myoclonic epilepsy	1(2.2%)	0 (0.0%)		

Mann-Whitney U and Chi-square tests were applied, ***: very highly significant. DRE: drug-resistant epilepsy; CE: Controlled epilepsy, HC: Healthy control.

Table 3: MiRNA-223 expression and serum HMGB1 and hs-CRP levels among the three studied groups.

Studied markers	DRE (n=46)	CE (N=25)	HC (N=25)	P value
miRNA223				
Mean ± SD	98.95 ± 41.61	24.9 ± 7.7	0.9 ± 0.3	**P^1<0.001 *** P^2<0.001*** P^3<0.001*** P^4=0.013***
Range	59.96 - 258.9	25.3 (11.2 - 38.4)	0.9 (0.4 - 1.4)	
HMGB1 (ng/ml)				
Mean ± SD	0.50 ± 0.10	0.21 ± 0.11	0.16 ± 0.08	**P^1<0.001*** P^2<0.001*** P^3<0.001** P^4=0.171
Range	0.48 (0.30 - 0.79)	0.21 (0.07 – 0.38)	0.15 (0.02 – 0.31)	
hs-CRP (mg/l)				
Mean ± SD	11.6 ± 6.7	1.9 ± 1.4	0.7 ± 0.5	**P^1<0.001*** P^2<0.001*** P^3<0.001*** P^4=0.639**
Range	9.9 (4.0 - 39.4)	1.4 (0.5 - 5.8)	0.7 (0.1 - 1.5)	

*: significant (p<0.05), ***: very high significance, One-way ANOVA test was applied.P^1: Comparison among all groups; P^2: Comparison between cases with DRE vs. cases with CE; P^3: Comparison between cases with DRE vs. healthy controls; P^4: Comparison between cases with CE vs. healthy controls, DRE: drug-resistant epilepsy, CE: controlled epilepsy, HC: healthy control.

Figure 1 showed a significant positive correlation between miRNA223 and HMGB1 (r=0.564, p<0.001), also, a significant positive correlation was observed between hs-CRP level and both miRNA (r=0.603, p<0.001), and HMGB1.
level (r=0.542, p<0.001).

Table 4 and **Figure 2** show the predictive ability of different studied markers for the detection of patients with epilepsy by using the ROC curve analysis. For miRNA-223 at a cut-off value of ≥ 6.3; the areas under the ROC curve was 1.0 with a sensitivity and specificity of 1.0% for both. For HMGB1 at a cut-off value of ≥ 0.27 (ng/ml); the areas under the ROC curve was 0.864, with a sensitivity of 81.2%, and a specificity of 92.0%. For hs-CRP at a cut-off value of ≥ 1.3 (mg/dl); the areas under the ROC curve was 0.944 with a sensitivity of 84.5%, and a specificity of 88.0%.

Table 5 and **Figure 3** show the predictive ability of different studied markers for the detection of the DRE by using the ROC curve analysis. For miRNA-223 at a cut-off value of ≥ 6.3; the areas under the ROC curve was 1.0 with a sensitivity and specificity of 100.0%. For HMGB1 at a cut-off value of ≥ 0.39 (ng/ml); the areas under the ROC curve was 0.991 with a sensitivity of 93.5.0% and a specificity of 100.0%. For hs-CRP at a cut-off value of ≥ 3.9 (mg/l); the areas under the ROC curve was 0.990 with a sensitivity of 100.0% and a specificity of 88.0%.

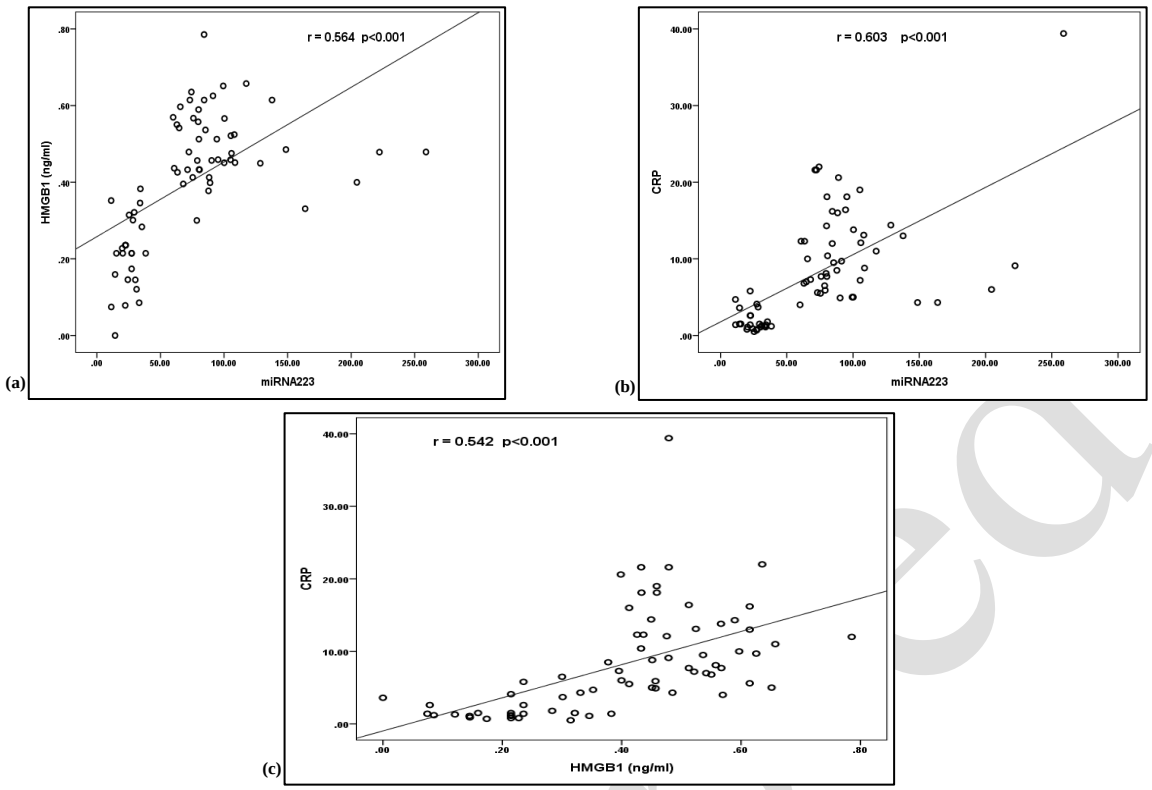

Fig. 1: (a) The correlation between plasma miRNA223 and HMGB1 in patients with epilepsy; (b) The correlation between plasma miRNA223 and hs-CRP in patients with epilepsy; (c) The correlation between plasma HMGB1 and hs-CRP in patients with epilepsy.

Table 4: The best cut-off, sensitivity, and specificity for detection of patients with epilepsy by studied biomarkers.

Markers	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	p-value
miRNA223	≥ 6.3	100.0%	100.0%	100.0%	100.0%	100.0%	1.0	**<0.001*** **
HMGB1(ng/ml)	≥0.27	81.2%	92.0%	97.7%	56.1%	84.1%	0.864	**<0.001*** **
CRP	≥ 1.3	84.5%	88.0%	95.2%	66.7%	85.4%	0.944	**<0.001*** **

PPV: positive predictive value; NPV: negative predictive value; AUC: Area under the curve; ***: very highly significant (p<0.001).

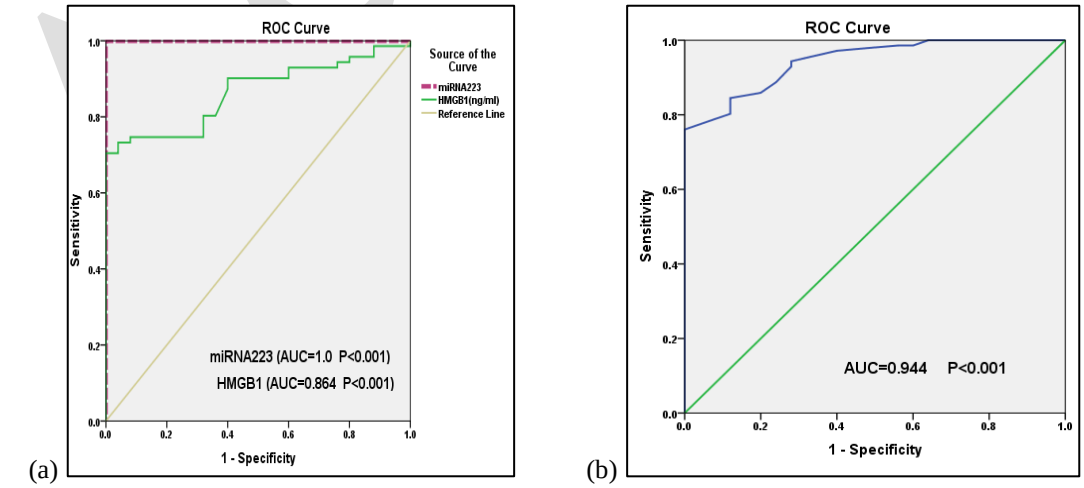

Fig. 2: (a) ROC curve of miRNA223 and HMGB1 for epileptic seizure detection in studied participants; b) ROC of hs-CRP curve for epileptic seizure detection in studied participants.

Table 5: The best cut-off, sensitivity, and specificity for detection of DRE by studied biomarkers

Markers	Cut- off	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	p-value
miRNA223	≥ 6.3	100.0%	100.0%	100.0%	100.0%	100.0%	1.0	<0.001***
HMGB1(ng/ml)	≥ 0.39	93.5%	100.0%	100.0%	89.3%	95.8%	0.991	<0.001***
hs-CRP	≥ 3.9	100.0%	88.0%	93.9%	100.0%	95.8%	0.990	<0.001***

PPV: positive predictive value; NPV: negative predictive value; AUC: Area under the curve, ***: very highly significant (p<0.001).

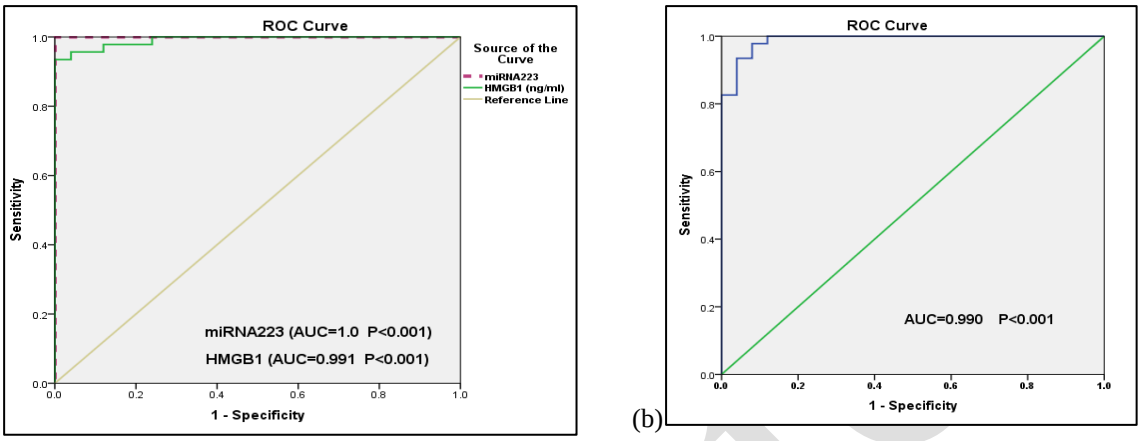

Fig. 3: (a) ROC curves of miRNA223 and HMGB1 for DRE detection in studied participants; (b) ROC curve of hs-CRP for DRE detection in studied participants.

DISCUSSION

In this study, we found that miRNA223 expression was considerably higher in patients with epilepsy in comparison with healthy controls (72.88 ± 49.01 vs. 0.93± 0.26, P<0.001). This increased level might reflect the role of dysregulated miRNA expression in mechanisms of epileptogenesis through inflammatory pathways, cell death, neuronal excitability, and synaptic reorganization, which underlie epileptogenesis[13].

Also, in the current study, miRNA-223 was significantly higher in patients with DRE than those with CE patients (98.9±41.6 vs. 24.9 ± 7.7, p<0.001). Thus, it has the best diagnostic value for epilepsy and DRE (100% sensitivity and 100% specificity) at a cut-off value≥ 6.3. This is consistent with a similar study carried out by De Beneditt and his coworkers[13], who conducted a study on a similar three groups (DER, CE patients, and healthy controls) using (miRNA-146, miRNA-142, and miRNA-223) as diagnostic biomarkers. They found that the 3 biomarkers were significantly upregulated in the patients with epilepsy compared to the healthy control subjects with (p< 0.05, p< 0.01, p< 0.001, respectively**),** and that miRNA-142 and miRNA-223 were significantly higher in patients with DRE than those with CE patients with (p<0.001).

The elevated level of miRNA-223 in DRE patients than CE may be due to the involvement of miRNAs in mechanisms of pharmacoresistance *MDR1* and *HIF-1α* overexpression, which lead to extrusion with resultant lower concentration of ASDs in brain cells[21]. Furthermore, another Turkish study was done on pediatric patients with DRE, CE, and healthy controls. It was reported that miRNA-223 and miRNA-155 were significantly elevated in epilepsy children than healthy children. Also, miRNA-223,miRNA-155, and miRNA-146 were significantly higher in children with DRE than in those with CE[22].

However, another study was carried out on children with febrile seizures reported increased miRNA-223 expression in patients with febrile seizures than controls, but the difference was insignificant[23].

There is mounting evidence that HMGB1 is a key player in neuroinflammation and mediates immunological and inflammatory responses in the central nervous system[15]. Inflammatory mediators have been shown to contribute to aberrant angiogenesis and impaired blood-brain barrier permeability, which are conditions closely linked to epileptogenesis[24].

However, unchecked focal or systemic inflammatory processes contribute to the process of epileptogenesis by causing abnormal neuronal connections, hyperexcitable neural networks, and a changed reaction to neurotransmitters[25].

In our study, we reported that patients with DRE had significantly higher levels of HMGB1 (P<0.001) in their blood compared to patients with CE or healthy controls (0.50 ± 0.10 vs. 0.21± 0.11 and 0.16 ± 0.08, respectively. Thus, it had diagnostic value for DRE with AUC 0.99, sensitivity 93.5%, and specificity 100%, indicating high power to predict drug resistance in patients with epilepsy. This is consistent with Walker[26] at Tampere University Hospital, Finland. who found that patients with DRE have significantly higher

HMGB1 levels than those of healthy controls and patients with CE (p=0.0001, AUC =0.99,sensitivity 93% and specificity 95%), this indicates that HMGB1 has an outstanding ability in distinguishing epileptic patients in suspicious cases and in predicting drug resistance to ASDs.

Furthermore, Viswas[27] in New Delhi, India, conducted a study on 50 patients with DRE and 50 patients with CE and revealed that HMGB1 was substantially raised in patients with DRE than those with CE ($p < 0.001$), with sensitivity 84% and specificity 80%.

Also, another study on children with epilepsy by Sakhr et al.[28] was carried out on 3 groups of children (patients with DRE, patients with CE, and healthy control) and demonstrated that HMGB1 was significantly higher in patients with epilepsy than healthy control ($P< 0.0001$) with AUC =0.97, sensitivity 90% and, specificity 93.75%,)moreover HMGB1 was considerably higher in patients with DRE than patients with CE ($P< 0.0001$) with AUC=0.95, sensitivity 88% and, specificity 96%).

The explanation of this result might be because the HMGB1-TLR4 axis contributes to the overexpression of P-gp, a BBB protein, which is induced in DRE foci and extrudes various ASDs from the brain. Thus, the collective evidence suggests that HMGB1 is implicated in epilepsy as a neuroinflammatory mediator which promotes epileptogenesis and is also implicated in pharmacoresistance and consequently in DRE[18].

Furthermore, in comparison of serum HMGB1 levels between patients with epilepsy and healthy control, it was found that HMGB1 levels were significantly higher in patients with epilepsy than healthy control group ($P<0.001$). This might indicate its importance as a diagnostic biomarker for epilepsy in suspicious cases, with 81.2% sensitivity and 92% specificity. Moreover, in agreement with our results, another study done by Kan[17] in China, demonstrated that HMGB1 was significantly higher in patients with epilepsy than in healthy control with a good predictive value of epilepsy risk with an AUC of 0.905 (95%CI, 0.864−0.946).

Moreover, the serum HMGB1 level of epileptic patients was considerably greater than that of matched controls ($P=0.0002$), according to a meta-analysis of 12 investigations[29].

The alterations in the brain in animal models of acquired epilepsy seem to be reflected in serum levels of HMGB1. In contrast to mice injected with anti-HMGB1 medications, anti-HMGB1 monoclonal antibodies, or lacking HMGB1-activated TLR4, mice injected intracerebrally with HMGB1 experience greater seizures in response to chemoconvulsant[15].

On the contrary, another study by Hautala[30] in Finland revealed no significant difference between patients with epilepsy and controls with respect to HMGB1. The explanation of these different results from the result of the current study may be due to the difference in the type and age group of patients, where Finland study was done on children with febrile seizure and matched control children with febrile infections but without febrile seizure.

In the current study, there was a significant positive correlation between miRNA-223 and HMGB1. This is in agreement with Olcum[31] at Izmir University, Turkey. This could refer to the importance of both miRNA-223 and HMGB1 as diagnostic biomarkers of DRE.

Furthermore, it has been revealed that hs-CRP is a main acute-phase reactant, especially for low-grade chronic inflammation, helpful as a risk factor in predicting various diseases in men as well as women. However, the relationship between the severity of the injury and CRP could not be determined precisely[32]. Serum levels of hs-CRP seem to be the most frequently studied inflammatory biomarker in people with epilepsy[33, 34].

In this study, the hs-CRP level in DRE patients was significantly higher in comparison to patients with CE and the healthy group ($P<0.001$). It had 100% sensitivity and 88% specificity for the diagnosis of DRE with an AUC of 0.99. This is in agreement but higher than the results of Demir et al. [35] study which found that serum hs-CRP levels of patients with resistant epilepsy were significantly higher ($p=0.026$**)** compared to CE patients (AUC 0.64, sensitivity 57.9 %, specificity 42.6 %). On the other hand, another prospective study was done to detect changes in hs-CRP in patients with DRE and revealed no significant difference in hs-CRP levels in patients with DRE[36].

In the current work, it was found that the hs-CRP level was significantly higher ($P<0.001$**)** in patients with epilepsy than in healthy controls. Its sensitivity as a diagnostic biomarker for epilepsy is 84.5% and 88% specificity, with an AUC of 0.94.

Furthermore, a meta-analysis of 16 studies involving 1,918 cases reported that epileptic patients had increased hs-CRP levels compared to controls[33]. This meta-analysis is followed by many studies that confirm it[37-41] which also demonstrated that hs-CRP was significantly higher in patients with epilepsy than in controls. This high level of hs-CRP may reflect the role of inflammation, either focal or systemic, which causes neural connectivity and hyper-excitability that participate in epileptogenesis[42].

In contrast, another study detected that there was no significant variance between the level of CRP in patients with epilepsy and controls. These different results may be due to the different patient groups, as the latter study was done on pregnant patients with epilepsy versus healthy pregnant women[43].

Also, there was a substantial positive relation between CRP and HMGB1 in this study, which is in agreement with previous studies that also found a

positive correlation between HMGB1 and CRP in patients with febrile convulsions and controls[44].

Results of the current study could pave the way for the presence of highly sensitive and specific biomarkers for confirming diagnosis of epilepsy in suspicious cases and predicting drug resistance as miRNA-223, HMGB1 and hs-CRP might be good biomarkers for diagnosis of epilepsy and prediction of drug-resistant with areas under the ROC curve (AUC= 1, 0.9, and 0.9, respectively). This can help in choosing a proper treatment option and open the door to research for the detection of new drugs for DRE.

CONCLUSION

In conclusion, the outcome of clinical trials of innovative medicines for the treatment or prevention of drug resistance is probably dependent on the identification of biomarkers that can identify individuals who are at risk of acquiring drug resistance. miRNA-223, HMGB1, and hs-CRP are biomarkers that are significantly increased in patients with epilepsy thus they are important biomarkers for non-invasive diagnosis of epilepsy in suspicious cases. They are also significant biomarkers for predicting DRE which may pave the way to the development of new antiepileptogenic drugs as anti-HMGB1, monoclonal antibodies, or inhibitors of miRNA-223 that can target the upregulated miRNA and inhibit epileptogenesis.

Recommendations

The limitation of this study is the small sample size, which needs to be larger for confirmation of this result, and this needs much more financial support. Also, difficulty in ascertaining the date of the last seizure in our patients, which leads us to choose the duration of the last seizure as more than 6 months for the controlled epilepsy group and less than 6 months for the DRE group.

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Conflicts of interest: All authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Declaration:

The manuscript is submitted for consideration for publication in the Egyptian Journal of Medical Microbiology. Please specify the category of the submitted manuscript.

The research contained in the manuscript has not been published, and the manuscript is not under consideration elsewhere

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