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RESEARCH

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Glutamate as a new path in discrimination between neuromyelitis optica spectrum disorder and multiple sclerosis

Amany Mahmoud Rabah[1], Mohamed El Sayed El Awady[1], Laila Ahmed Rashed[2], Doaa Abdellatif Elelwany[1,3*] and Al-Shaimaa Mahmoud Aboul Fotouh[1]

Abstract

Background Neuromyelitis optica spectrum disorder (NMOSD) used to be considered as a variant of multiple sclerosis (MS), however the recent detection of a highly specific serum biomarkers for NMOSD have made clear that NMOSD is a condition distinct from MS. The aim was to explore the role of serum glutamate level in the discrimination between NMOSD and relapsing remitting (RR) MS patients during and in between relapses. The study comprised two groups; first group, a total of 30 NMOSD patients, they were furtherly subdivided into NMOSD in remission, 15 patients without recent relapses in the last 3 months, NMOSD with relapse, 15 patients with recent relapses in the last 3 months, the second group, 30 definite, RRMS patients, they were further subdivided into RRMS in remission, 15 patients without recent relapses in the last 3 months RRMS with relapse, 15 patients with recent relapses in the last 3 months.

Results Without relapse, NMOSD patients have higher level of serum glutamate than RRMS patients with (*P* values = 0.005), a significant difference between EDSS in NMOSD patients and RRMS patients (*P* = 0.0001), The cut-off value of glutamate serum level between NMOSD in remission and RRMS in remission was > 10.3 µg/mL, yet its level for differentiation between group RRMS in remission and RRMS with relapse was > 12.6 µg/mL.

Conclusion Glutamate cut-off value might be a reliable tool to discriminate between NMOSD and RRMS.

Background

Neuromyelitis optica spectrum disorder (NMOSD) and multiple sclerosis (MS) are autoimmune inflammatory demyelinating diseases of the central nervous system (CNS) and distinct etiology presenting with optic neuritis (ON) [1]. Although distinguished by clinic-radiological and demographic features, early manifestations can be similar complicating management [2].

NMOSD has a distinct immunopathogenesis being a primary autoantibody mediated astrocytopathy, it includes limited forms of NMOSD, such as isolated recurrent ON, longitudinally extensive TM (LETM) [3]. There are three cardinal manifestations in NMOSD, transverse myelitis, optic neuritis, and area postrema syndrome. The disability in NMOSD is associated with poor recovery and the lack of a progressive phase [4].

NMOSD can be distinguished from MS through a combination of clinical findings, imaging investigations, and serological analysis. The interaction between the nervous and immune systems involves an important role for neurotransmitters, heterogeneity of the pathogenetic processes in MS is evidence for impairment of this link.

*Correspondence:
Doaa Abdellatif Elelwany
doaa.abdellatif@outlook.com
[1] Neurology Department, Kasr Al Ainy Hospital, Faculty of Medicine, Cairo University, Giza, Egypt
[2] Biochemistry Department, Faculty of Medicine, Cairo University, Giza, Egypt
[3] 3th Omar Khatab st, Nazleh El Saman, El Haram, Giza, Egypt

Glutamate is the most important of these, as well as the most intensely studied in recent years [5, 6], Glutamate, a neurotransmitter, at normal level performs crucial processes like memory and sensory perception [7].

Glutamate is made by nerve cells and is stored in thin-walled vesicles called synaptic vesicles located at the axon terminal. Each vesicle can contain thousands of neurotransmitter molecules. Glutamate controls CNS homeostasis. It determines the principal neuronal property involved in the ability of CNS to resist insults, to assure an efficient neuronal response to stimuli and to build up restorative adaptations. Glutamate excess can trigger a cascade of oxidative reactions in the brain leading to many neurologic diseases such as MS, amyotrophic lateral sclerosis, epilepsy, and secondary injury following stroke [8, 9].

In Alfredsson et al. [10] found that the serum levels of glutamate were significantly positively correlated with the CSF level in healthy volunteers ($r=0.67$, $P<0.05$) which indicates the relationship between the metabolism of glutamate in brain and peripheral tissues. In addition, many studies reported BBB dysfunction in multiple sclerosis and NMOSD which will increase such correlation [11–13].

The aim of this study is to investigate the level of serum glutamate in NMOSD and RRMS during both relapses and remissions.

Methods

This is a case control, single-center study. NMOSD and RRMS patients were selected consecutively. A total of 30 NMOSD patients (group 1); aged between 18 and 60 years who fulfilled the revised diagnostic criteria of the International Panel for NMOSD Diagnosis (IPND) [14], and 30 RRMS patients (group 2) diagnosed according to revised McDonald criteria 2017 [15], subjects were recruited from Kasr Al Ainy MS clinic, Cairo University hospital; Cairo University, during the period from September 2019 till April 2020.

Patients were divided into two groups; group 1 included the thirty NMOSD patients, they were further subdivided to, 15 patients without recent relapses in the last 3 months [16] and 15 patients with recent relapses in the last 3 months. Group 2, 30 RRMS patients, they were further subdivided into 15 MS patients without recent relapses in the last 3 months and 15 patients with recent relapses in the last 3 months [16]. Sample size was done using G* power data analysis software. Total sample size is 56 when the actual power is 0.95 based on a pilot study. Exclusion criteria for NMOSD and RRMS patients were: history of corticosteroid intake within 3 months prior to the study, and patients with general medical conditions such as hepatic disease. Demographic characteristics and clinical data as age, sex, age of onset, duration of illness, full neurological examination, expanded disability status scales (EDSS) were recorded for each patient; and in the groups of the relapsed patients, the date of onset and maximum worsening was defined, EDSS was calculated at the day of the maximum worsening [17].

The severity of the relapses was measured by the difference between baseline EDSS and the EDSS at maximum symptom worsening and was graded as mild if EDSS increases by 0.5–2 points, moderate if EDSS increases by 2.5 or 3 points and severe if EDSS increases by ≥ 3.5 points [18]. All patients were subjected to; blood samples by median cubital venipuncture, 2 mL were collected and stored at − 20 °C and tested for glutamate by enzyme-linked immunosorbent assay (ELISA).

Principle of the test: quantitative determination of glutamate after extraction and derivatization by ELISA. The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. 25 µL of the prepared calibrators, controls and unknowns were pipetted into the appropriate wells of the glutamate microtiter strips. The acylated calibrators, controls and unknowns and the solid phase bound analyte compete for a fixed number of antiserum binding sites. The concentrations of plasma glutamate have been read in the wells within 10 min, using a microplate reader set to 450 nm and a reference wavelength between 620 and 650 nm.

Statistical analysis, normality of data was tested by the Kolmogorov–Smirnov test. MedCalc version 18.11.6 was used for statistical analysis. Numbers and percentages represented qualitative data and Chi-square tested for proportion independence. Unpaired *t*-tests were used for comparing mean values of two independent groups. The *P*-value was always two-tailed and considered significant at the 0.05 level. A receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was analyzed to detect the best cut-off value for serum glutamate to differentiate NMOSD patients in remission and RRMS patients in remission, also to differentiate between RRMS patients in remission and RRMS patients with relapse. The Pearson correlation coefficient—also known as Pearson's *r*—was used to measure the linear correlation between glutamate serum level and different clinical parameters.

Results

In NMOSD 27 patients (90%) were females, while out of 30 RRMS patients 21 (70%) were females. In RRMS patients, there is a significant difference between both groups regarding the age at disease onset ($P=0.004$). On the other hand, no significant difference between both

groups regarding age, duration of illness and the duration between relapse to sampling (Table 1).

The EDSS of NMOSD patients ranged from 1.5 to 7 with median of 4.5, while that of RRMS patients ranged from 1 to 5.5 with median of 2.5, with a significant difference, ($P=0.0001$). The most commonly affected domains in NMOSD patients were pyramidal (83.3%) then visual (76.7%) while in RRMS patients were pyramidal (96.7%), then sensory (63.3%) with a significant difference between both groups ($P<0.0001$) (Fig. 1).

Regarding NMOSD patients with relapse, among 15 patients, relapses were monosymptomatic in 6 (40%); visual in all of them (100%), while they were poly-symptomatic in 9 patients (60%); all of them (100%) had motor, sensory and bladder symptoms. While among 15 RRMS patients with relapse, relapses were monosymptomatic in 9 patients (60%); visual in 5 (55.6%) patients, and brainstem in 2 (22.2%) and motor in 2 (22.2%), while they were poly-symptomatic in 6 patients (40%); 4 (66.7%) of patients had motor symptoms, 3 (50%) had sensory, 3 (50%) had cerebellar symptoms, 2 (33.3%) had visual, 2 (33.3%) had brainstem symptoms, and 1(16.7%) had bladder symptoms. The Severity of attacks

Table 1 Clinical characteristics and glutamate serum level among study groups

	NMOSD ($n=30$)				**MS ($n=30$)**			
	Patients with relapse ($n=15$)		**Patients in remission ($n=15$)**		**Patients with relapse ($n=15$)**		**Patients in remission ($n=15$)**	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Age	25–57	38.4 ± 7.68	19–53	38 ± 9.06	24–43	35 ± 6.4	26–45	33.2 ± 5.8
Age at onset (years)	21–48	32. 73 ± 7. 72	19–49	33.2 ± 10.8	18–36	26 ± 7.06	18–37	26.53 ± 6.38
Duration of illness	5 m–15 y	5.17 ± 4.11 y	5 m–16 y	4.8 ± 4.6 y	10 m–16 y	7.03 ± 4.76	6 m–11 y	5.36 ± 4.36
Last relapse to sampling duration	4 m: 63 m	29.4 ± 22.8 m	1 day: 3 m	1.02 ± 0.9 m	3.47 m: 87 m	16.6 ± 21.35 m	1: 31 days	13.3 ± 11.6 days
	Range	**Median**	**Range**	**Median**	**Range**	**Median**	**Range**	**Median**
Total number of relapses	02-Jun	3	01-Nov	3	01-Oct	3	01-Nov	6
Number of relapses in the last year	0–4	1	01-Mar	2	0–4	1	1–5	3
Total EDSS	1.5–7	4	02-Jul	5	01-May	2.5	1.5–5.5	2.5
	Range	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**	**Range**	**Mean ± SD**
Glutamate (µg/mL)	8.2–33.6	16.82 ± 6.98	5.09–34.2	18.46 ± 8.76	5.3–17.5	10.84 ± 3.26	10.8–38.4	23.69 ± 8.64

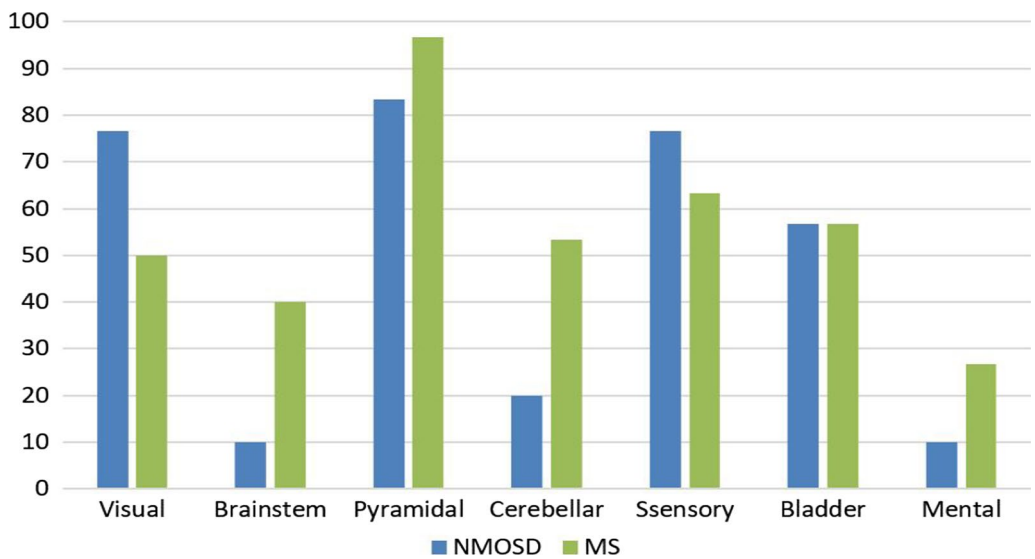

Fig. 1 Frequency of symptoms in NMOSD and RRMS patients

between both studied groups showed no statistically significant difference ($P=0.6$).

While in NMOSD patients in remission no new T2 lesions or contrast enhanced lesions were observed in the last MRI brain, four patients out of fifteen in NMOSD patients with relapse showed T2 lesions in the last MRI and no contrast enhanced lesions were observed. In RRMS patients in remission, the last MRI showed T2 lesions in 15 (100%) and none of them showed enhanced lesions. In RRMS patients with relapse, new T2 lesions were observed in 15 (100%).

MRI spine in NMOSD patients in remission showed lesions more than 3 vertebral segments in 12 (80%) patients, less than 3 vertebral segments in 1 (6.7%). In NMOSD patients with relapse, it showed lesions more than 3 vertebral segments in 9 (60%) patients, less than 3 vertebral segments in 4 (26.7%). In RRMS patients in remission, MRI cervical spine showed lesions less than 3 vertebral segments in 8 (72.7%) patients, more than 3 vertebral segments in 1 (9.1%). RRMS patients with relapse, it showed lesions less than 3 vertebral segments in 7 (70%) patients.

Glutamate level

The level of glutamate was found to be significantly higher in NMOSD patients in remission 16.82 ± 6.98 µg/mL when compared with RRMS patients in remission 10.84 ± 3.26 µg/mL (P value = 0.005); however, no significant difference was found between NMOSD patients with relapse 18.46 ± 8.76 µg/mL and RRMS patients with relapse 23.69 ± 8.64 µg/mL (P value 0.1).

A strong significant negative correlation was found between glutamate serum level and period from last relapse to sampling in NMOSD patients with relapse, ($R=-0.822$, $P=0.0001$) (Fig. 2).

Also, a significant positive correlation was found between glutamate level in RRMS patients in remission and total number of relapses, EDSS, and total duration of disease ($R=0.634$, 0.615,0.57, $P=0.01$, 0.01, 0.02, respectively) (Figs. 2, 3).

The cut-off value of glutamate serum level for differentiation between NMOSD in remission and RRMS in remission was > 10.3 µg/mL with (P value = 0.0009), while the cut-off value of glutamate serum level for differentiation between RRMS in remission and RRMS with relapse was > 12.6 µg/mL with (P value < 0.0001) (Table 2).

Discussion

Starting with clinical data as a clue to differentiate NMOSD patients from RRMS patients; NMOSD patients are older than RRMS patients at the onset of illness that is in line with Pandit and colleagues [19], while in a more recent study conducted by Lucía Romero-Pinel et al. [20] the age at onset RRMS has increased over the last five decades.

Regarding clinical severity and disability data, our results demonstrated that NMOSD patients had higher EDSS than RRMS patients with a significant P value = 0.0001, these results are in agreement with Juryńczyk et al. [21].

Comparing the most common affected domain in NMOSD and RRMS patients, we found that pyramidal

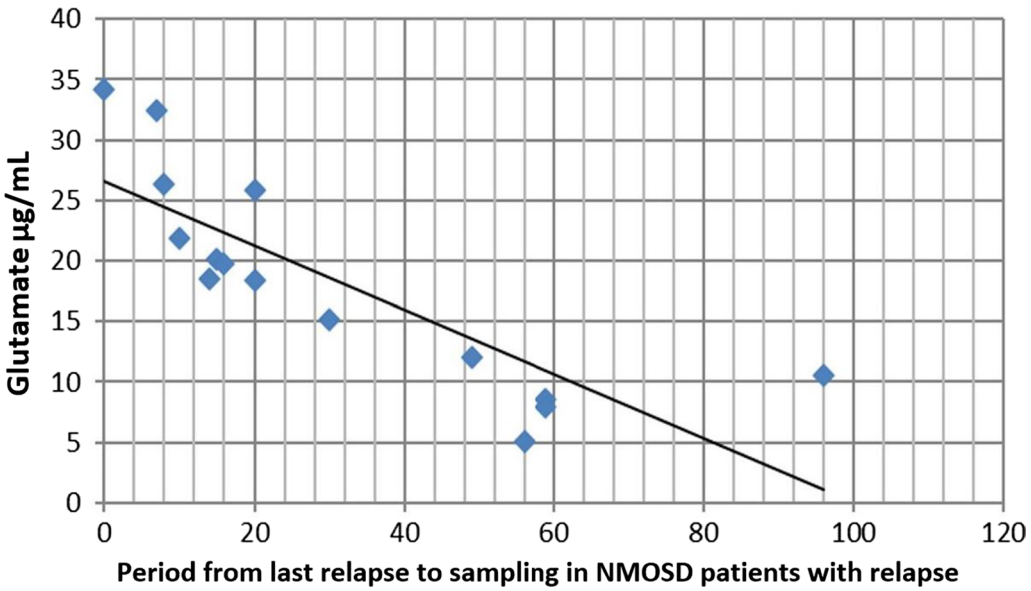

Fig. 2 Correlation between glutamate serum level and period from last relapse to sampling in NMOSD patients with relapse

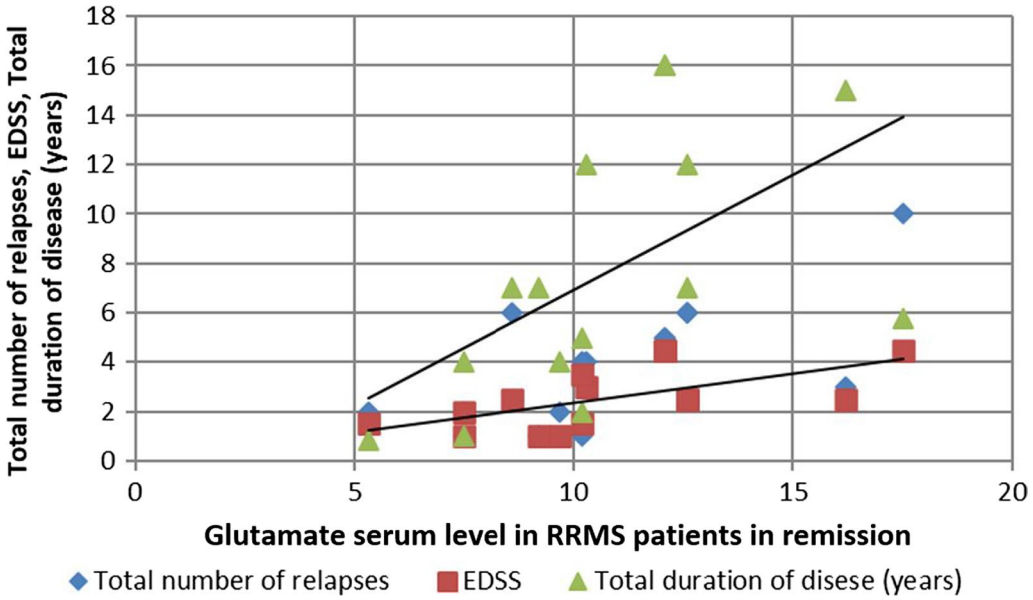

Fig. 3 Correlation between total number of relapses, EDSS, total duration of disease and glutamate serum level in RRMS patients in remission

Table 2 Estimation of glutamate level cut-off value

	Area under the curve	*P* value	95% Confidence interval		Cut-off value µg/mL (mm)	Sensitivity (%)	Specificity (%)
			Lower bound	Upper bound			
NMOSD in remission versus RRMS in remission	0.782	0.0009	0.594	0.911	> 10.3	86.67	60
RRMS in remission versus RRMS in relapse	0.944	< 0.0001	0.795	0.995	> 12.6	93.33	80

was the most affected domains in both (96.7% and 83.3%, respectively) while 76.7% of NMOSD patients had visual affection on the other hand 50% of RRMS patients had visual affection, there was a high significant difference between both groups, $P < 0.0001$. Many studies supported our finding [22, 23].

For the second time in our study, our results revealed that the burden of illness in NMOSD is greater than that in RRMS as in NMOSD with relapse, among 15 patients, relapses were poly-symptomatic in 60% of patients and monosymptomatic in 40%. While among 15 patients in RRMS with relapse, relapses were monosymptomatic 60% and poly-symptomatic 40%.

Coming to radiological findings among our participants; in NMOSD patients with relapse, only four patients showed T2 lesions in the last MRI brain on the other hand, all RRMS patients in remission, showed T2 lesions in MRI brain and all patients in group RRMS with relapse showed new lesions.

Eighty presents of NMOSD group a patients had MRI spine lesions more than three vertebral segments in comparison to 9.1% of patients in RRMS in remission.

Regarding multiple sclerosis, disruption of the BBB is one of the underlying pathogeneses, which follows massive infiltration of T cells forming demyelinated plaques. Dysfunction of the BBB is also reported in neuromyelitis optica spectrum [24]. When anti-AQP4-IgG obtained from a NMOSD patient and administered to mice, lesions of perivascular astrocyte were noticed, indicating the dysfunction of BBB by impairing the expression of tight junction proteins [25].

Our results matched with that Collins and colleagues and Filippi et al. [26, 27] who proved the utility of MRI in demyelinating disease diagnosis.

To our knowledge this is the first study to compare serum glutamate level between NMOSD and RRMS patients, and shows that the level of glutamate was significantly higher in NMOSD patients without relapses

when compared to MS patients without relapses with $P=0.005$, it is a cheaper, easy and less invasive adjuvant tool for differentiation that may be of help especially in cases of AQ4 negative NMOSD; however, no significant difference was found between NMOSD and RRMS patients with relapses.

The higher glutamate level in NMOSD patients in remission could be related to the marked impairment of glutamate uptake by astrocytes found in NMOSD, and this NMOSD astrocytopathy is distinguishable from the immunopathology of multiple sclerosis.

Al Gawwam et al. [28] found a significant increase in serum glutamate in MS patients without relapse when compared to healthy controls. Other MS studies have also demonstrated increased levels of glutamate in CSF [29] or by using MRS [30]. The involvement of glutamate in the pathogenesis of MS could represent a secondary pathophysiological mechanism leading to demyelination and neuroaxonal damage.

As glutamate level will decline over time that might explain the presence of significant difference of serum glutamate between RRMS patients with and without relapses and the absence of such difference between NMO patients with and without relapses and between NMOSD patients with relapses and RRMS patients with relapses.

In RRMS patients without relapses, there was a significantly positive correlation between glutamate serum level and EDSS ($r=0.615$, $P<0.05$), but no such correlations were found in MS patients with relapses. This finding is consistent with an earlier study in Westall et al. [31].

Furthermore, the study in [30], which used MRS to evaluate the contribution of glutamate toxicity in MS, showed a correlation between increased levels of glutamate and EDSS, as they found that excess glutamate was associated with accelerated rate of neuroaxonal integrity, brain volume loss and worsening of clinical outcomes.

Using receiver operating characteristic (ROC) curve and AUC, the cut-off value of glutamate serum level for differentiation between NMOSD patients in remission and RRMS patients in remission was > 10.3 µg/mL with sensitivity 86.7% and specificity 60%, and the cut-off value of glutamate serum level for differentiation between RRMS patients in remission and RRMS patients with relapse was > 12.6 µg/mL with sensitivity 93.33% and specificity 80% (P value < 0.001). Validity analysis in another study stated that cut-off values of 17.5 µg/mL for glutamate and 75.2 nmol/mL for nitric oxide can predict occurrence of relapse [32].

Conclusions

Diagnostic work-up and monitoring of NMOSD and RRMS can include serum glutamate regular assessment for two purposes: first, to improve diagnosis and prognosis of the course of illness; and second, to identify true relapses and differentiate them from clinical worsening of pre-existing symptoms in RRMS.

Abbreviations

AQP4	Aquaporin-4
AUC	Area under the curve
BBB	Blood brain barrier
CNS	Central nervous system
CSF	Cerebrospinal fluid
EDSS	Kurtzke Expanded Disability Status Scale
ELISA	The enzyme-linked immunosorbent
NMOSD	Neuromyelitis optica spectrum disorder
MS	Multiple sclerosis
RRMS	Relapsing remitting multiple sclerosis

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Author contributions

AMR was the idea founder, shared in the patient collection, and the supervisor in all the steps; MEE shared in the patient collection and supervision; DAE shared in the patient collection and is the submitting and corresponding author; LAR did the laboratory work; AMA did the data analysis, shared in the patient collection, wrote and revised the manuscript; All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are not publicly available due to current Cairo University regulations and Egyptian legislation but are available from the corresponding author on reasonable request and after institutional approval.

Declarations

Ethics approval and consent to participate

The research Ethics Committee (REC) of faculty of medicine, Cairo. University approved this study, code: MD-176-2019. informed written consents was obtained from the patients for participation in the study.

Consent for publication

Not applicable.

Competing interests

The author declared that they have no competing interest.

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