ASSESSMENT OF CHLORIDE INTRACELLULAR CHANNEL PROTEIN-1 (CLIC1) ANTIBODY AS A BIOMARKER IN THE DIAGNOSIS OF MULTIPLE SCLEROSIS (MS): A-PILOT CASE-CONTROL STUDY FROM EGYPT

Lamees A. Samy[1], Dalia Labib[1]*, Mona Nada[2], Noha Ramadan[1] and Diana Khedr[2]

[1]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, [2]Department of Neurology Faculty of Medicine, Cairo University

Abstract

Background: Multiple sclerosis is a chronic inflammatory disorder of the central nervous system. This study aimed to assess the association between the serum level of chloride intracellular channel 1 (CLIC1) antibody and the potential role of it in the diagnosis of MS.

Methods: We con-ducted a pilot case-control study involving 90 participants divided into three groups: relapsing remitting multiple sclerosis (RRMS) (n=49), secondary progressive multiple sclerosis (n=11), and healthy controls (n=30). Detailed disease characteristics using Expanded Disability Status Score (EDSS) and imaging biomarkers were assessed. Serum levels of chloride intracellular channel protein-1 (CLIC1) antibody were measured.

Results: The study showed statistically significant difference of Anti-CLIC1 levels when compared between the three groups including RRMS, SPMS and control groups. In the RRMS group, the mean Anti-CLIC1 level was 542.2. In the SPMS group, the mean Anti-CLIC1 level was 409.9. In contrast, the control group had a higher mean Anti- CLIC1 level of 585.0. The data examines the correlation between Anti-CLIC1 levels and three variables: age, duration of illness, and EDSS scores showing weak negative correlation between the three variables and the Anti- CLIC1 level with no statistically significant difference. ROC analysis showed that Anti-CLIC1 can significantly predict MS with AUC of 0.701, p-value < 0.001 with sensitivity of 56.7% and specificity of 86.7%

Conclusions: Considering the reduced levels of CLIC1-antibody in MS patients compared to the control group, it is tempting to hypothesize An-ti-CLIC1 is a fair diagnostic biomarker for diagnosis of multiple sclerosis.

Keywords: Multiple Sclerosis; EDSS; Anti- CLIC1; Neuroinflammation; Fluid Biomarkers.

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*Corresponding Author: Lamees A. Samy, Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University

Correo-e: labibdalia@yahoo.com

Introduction

Multiple sclerosis (MS) is autoimmune disease characterized by disruption of myelin sheath and categorized into two main types according to the clinical course: relapsing or progressive type. The relapsing-remitting multiple sclerosis (RRMS) is more common with partial or complete re-mission. After the disease onset, the relapsing form may develop into secondary progressive multiple sclerosis (SPMS). Few patients may undergo progression from the onset of the disease, a subtype termed primary progressive multiple sclerosis (PPMS) [1,2].

Multiple risk factors may contribute to the disease development. This involves genetic predisposition, host immune re-sponse, environmental triggers like Epstein-Barr virus, and exposure to chemicals or toxins. However, the exact cause of multiple sclerosis is still unknown [3]. The process of diagnosis typically involves evidence of inflammatory CNS injury with clinical symptoms that must last for >24 hours and occur as distinct episodes separated by at least 1 month, the main tests conducted are magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis according to the revised McDonald 2017 criteria [2]. The disease is becoming more prevalent worldwide, reaching from 2.1 million in 2008 to 2.3 million in 2013 [4] . Chloride intracellular channel protein-1 (CLIC1) anti-body is an autoantibody that may have a potential role in the diagnosis of MS. However, more research is still needed to fully understand the relationship between CLIC1 and MS [5] . We therefore sought to evaluate the serum levels of CLIC1 antibody in MS patients and correlate them to the serum levels of CLIC1 in healthy control in a case-control study.

Materials and Methods

In this case-control study, we retrospectively recruited 60 patients with MS diagnosis according to revised McDonald criteria 2017 during the period from November 2023 to February 2024. The pa-tients had been recruited from Kasr Al-ainy Multiple Sclerosis Unit (KAMSU) at Cairo University Hospital. 90 participants divided into three groups: relapsing remitting multiple sclerosis (RRMS) (n=49), secondary progressive multiple sclerosis (n=11), and healthy controls (n=30). Adults aged over 18 years and less than 60 years with MS diagnosis according to revised McDonald criteria 2017 were included [6]. We excluded patients diagnosed with other autoimmune diseases, pa-tients who received steroids in the last 30 days and patients who didn't give consent to participate in the study. All patients underwent thorough medical and neurological evaluations using EDSS score. Neuroradiological assessment using MRI scans of the brain and spinal cord were per-formed on a 1.5 Tesla, MRI scanner according to a standard protocol including T1-weighted axial scans with and without application of gadolinium-DTPA (Gd) as well as T2-weighted turbo in-version recovery with frequency-selective fat saturation (FLAIR) coronal scan. Laboratory anal-yses included measurement of Complete blood count (CBC), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) , C-reactive protein (CRP), Creatinine, sodium and potassium , Erythrocyte sedimentation rate (ESR) and Serum Anti-Chloride Intracellular Channel Protein 1 Antibody (Anti- CLIC1) using an Enzyme-Linked Immunosorbent Assay (ELISA) kit lot number 20240510 (SUNLONG biotech) .

Statistical analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA).

Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruska-Wallis and Mann-Whitney tests [7]. For comparing categorical data, Chi-square ($\chi 2$) test was performed. The exact test was used instead when the expected frequency is less than 5 [7]. Correlation between quantitative variables were done using the Spearman correlation coefficient [7]. The ROC curve was constructed with the area under curve analysis performed to detect the best cut-off value of Anti-CLIC1 for the detection of cases. P-values less than 0.05 were considered statistically significant.

Results

Demographic characteristics

The study involved 60 participants, with a majority being females (76.7%, 46 participants) and the rest were males (23.3%, 14 participants). Most of the participants were diagnosed with Re-Lapsing-Remitting Multiple Sclerosis (RRMS) (81.7%, 49 participants), while a smaller group had Secondary Progressive Multiple Sclerosis (SPMS) (18.3%, 11 participants). Regarding their current medications, 53 were under treatment and 11.7% (7 participants) were not using any treatment (Table 1). The average age of participants was 32.87 years, the duration of illness averaged 7.10 years, the EDSS scores had a mean of 2.84 and anti-CLIC1 levels showed a mean of 517.92 (Table 2).

The study compared Anti-CLIC1 levels across three groups: RRMS, SPMS, and control. For the RRMS group, the mean Anti-CLIC1 level was 542.2 with a standard deviation of 403.0. The SPMS group had a mean level of 409.9 with a standard deviation of 62.3. The control group showed a mean Anti-CLIC1 level of 585.0 with a standard deviation of 208.3. The difference among these groups was statistically significant, with a P value of 0.002. (Table 3)

Correlation between Anti-CLIC1 levels and three variables: age, duration of ill-ness, and Expanded Disability Status Scale (EDSS) scores

The data examines the correlation between Anti-CLIC1 levels and three variables: age, duration of illness, and Expanded Disability Status Scale (EDSS)

Table 1. Descriptive statistics of gender, type of MS, and medication among patients.

		Count	%
Sex	**Female**	46	76.7%
	Male	14	23.3%
Diagnosis	**RRMS**	49	81.7%
	SPMS	11	18.3%
Current medication	**Under treatment**	4	88.3%
	No treatment	7	11.7%

Table 2. Descriptive statistics of age, duration of disease, EDSS and Anti-CLIC1 among patients.

	Patients				
	Mean	**Standard Deviation**	**Median**	**Minimum**	**Maximum**
Age	32.87	8.72	32.00	19.00	56.00
Duration ofillness	7.10	5.17	5.50	1.00	22.00
EDSS	2.84	2.05	2.00	0.00	8.00
Anti CLIC1	517.92	368.06	438.80	268.60	3032.00

Table 3. Comparison of Anti-CLIC1 according to different types of MS.

	RRMS					SPMS					Control					Pvalue
	Mean	**SD**	**Median**	**Minimum**	**Maximum**	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**	**Mean**	**SD**	**Mdian**	**Minmum**	**Maximum**	
Anti CLIC1	542.18	403.03	438.80	268.60	3032.00	409.87	62.28	429.80	322.30	523.80	584.97	208.32	483.55	402.90	1231.00	0.002

Table 4. Correlation between Anti-CLIC1 levels and three variables: age, duration of illness, and Expanded Disability Status Scale (EDSS) scores.

		Anti-CLIC1
Age	**Correlation Coefficient**	-0.061-
	P value	0.644
	N	60
Duration of illness	**Correlation Coefficient**	-0.085-
	P value	0.519
	N	60
EDSS	**Correlation Coefficient**	-0.098-
	P value	0.456
	N	60

Table 5. ROC analysis showing the prediction of patients using Anti-CLIC1.

Area Under theCurve	**P value**	**95% Confidence Interval**				
		Lower Bound	**Upper Bound**	**Cut off**	**Sensitivity %**	**Specificity%**
0.701	< 0.001	0.594	0.807	< 441	56.7	86.7

scores in a sample of 60 individuals. Age and Anti-CLIC1 showed a correlation coefficient -0.061, suggesting a very weak negative correlation between Anti-CLIC1 levels and age. Duration of illness showed a correlation coefficient is -0.085, indicating a slightly stronger but still weak negative correlation between Anti-CLIC1 levels and the duration of illness. Anti-CLIC1, and EDSS showed a correlation coefficient -0.098, signifying a weak negative correlation between Anti-CLIC1 levels and EDSS scores (Table 4).

ROC analysis showing the validity of Anti-CLIC1 in MS patients

The ROC (Receiver Operating Characteristic) analysis presents an evaluation of the diagnostic performance of a test. The Area Under the Curve (AUC) is 0.701, indicating a fair level of accuracy in distinguishing between the conditions tested. The P value is less than 0.001, signifying that the test's performance is statistically significant. The chosen cut-off value for the test is less than 441, which results in a sensitivity of 56.7% and a specificity of 86.7%. This implies that the test correctly identifies 56.7% of true positive cases and correctly 86. 7 % of true negative cases at this cut - off point (Table 5) (Figure 1).

Discussion

Multiple sclerosis (MS) is an autoimmune disorder characterized by inflammation that attacks the myelinated axons in the central nervous system (CNS), causing damage to both the myelin and axon to different extents [8]. Chloride Intracellular Channel Protein 1 (CLIC1) is a member of the chloride intracellular channel protein family, expressed in microglia, the resident immune cells of the central nervous system, and is implicated in the production of reactive oxygen species (ROS) and the release of pro-inflammatory cytokines [9]. There is limited evidence on the role of CLIC1 antibody as a diagnostic biomarker for multiple sclerosis, thus we conducted a case- con-troll study to find an association between CLIC1 antibody as a biomarker in the diagnosis of multiple sclerosis. Our study showed that 76.7% of the patients were females, while 23.3% of the patients were males. These findings are consistent with several studies that indicated a higher incidence of MS among females compared to males accounting for a gender ratio 2.3–3.5:1 [10]. The average age of participants was 32.87 ±8.72 years, ranging between 19 and 56 years, with a mean duration of illness averaging 7.10 years. The EDSS scores had a mean of 2.84. Concerning the age our findings are consistent with the vast majority of studies, which indicated a mean age of diagnosis with MS 31.11 ± 9.82 years [11]. The suppression of CLIC1 chloride current or the downregulation of CLIC1 protein demonstrated a neuroprotective effect in a co-culture of neurons and microglia in the presence of beta-amyloid peptide [12]. The Anti- CLIC1 in the patient group was significantly lower among MS patients compared to controls with p-value of 0.002. SPMS showed significantly lower levels of anti- CLIC1 compared to controls with p-value of 0. 002.When measuring CLIC1 antibodies, no significant difference was observed between patients receiving treat-ment and those not on any medications, with a p-value of 0.787.

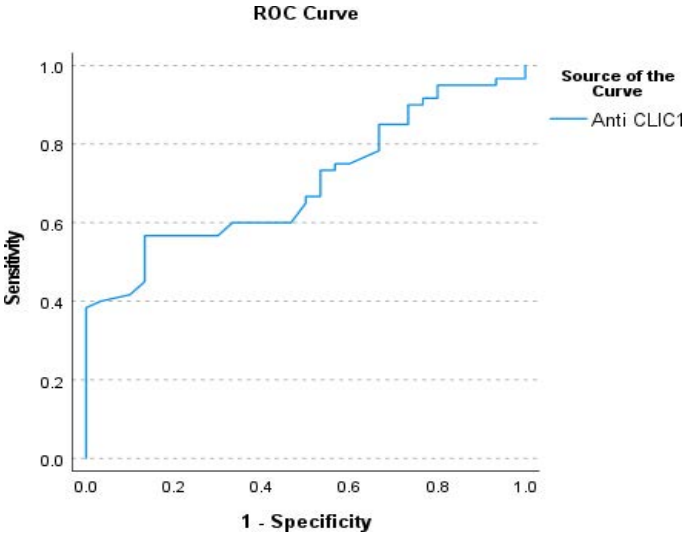

Figure 1. ROC curve for prediction of patients using Anti - CLI C.

Karaaslan et al conducted a cross-sectional analytical study involving patients with multiple sclerosis who primarily experienced spinal cord and optic nerve attacks (MS-SCON), patients with neuromyelitis optical spectrum disorder (NMOSD), RRMS patients with minimal or no history of myelitis/optic neuritis, re-lapsing inflammatory optic neuritis (RION) as well as healthy controls. They found CLIC1 anti-bodies in 31% of MS- SCON patients and 20% of those with RION. However, none of the NMOSD patients, RRMS, or healthy controls had these antibodies. Additionally, patients with CLIC1 anti-bodies tended to have lower disability scores [13]. Contrary to our study, it was suggested that Anti-CLIC1 may not be correlated with EDSS and disease severity, a possible explanation for the differing results could be due to relying on baseline EDSS without longitudinal follow-up may not reflect changes in disease progression over time. In the current study, ROC analysis showed that Anti-CLIC1 can significantly predict MS with AUC 0.701, p-value < 0.001 using cut-off value < 441, with sensitivity 56.7% and specificity 86.7%. The cut-off value of Anti-CLIC1 is equal to 441 ng/L for diagnosis of Multiple sclerosis. In the study conducted by Karaaslan et al., they identified the cut-off level for CLIC1 antibody positivity (48.3 pg/µl) as two standard deviations above the mean of healthy controls [13].A more likely explanation could be the use of different ELISA methods and reagents for the detection of Anti-CLIC1 resulting in different sensitivi-ties. Considering the reduced levels of CLIC1-antibody in MS patients compared to the control group, it is tempting to hypothesize that the production of CLIC1-antibody serves as a compensatory mechanism to counteract the pro-inflammatory effects of A1 astrocytes[13].The presence of Anti-CLIC1 in both patients and healthy controls indicates that CLIC1 is recognized by the immune system in both neurodegeneration and in normal states. This might be comparable to the body's compensatory mechanism observed in hepatitis B surface antigen. In the case of hepatitis B, a well-established threshold exists, where antibody levels of 10 milli-international units per milliliter (mIU/mL) or greater are considered protective [14]. However, the threshold at which CLIC1 antibodies may be considered protective is unclear. This research aimed to provide a more precise understanding of the role of Chloride Intracellular Channel Protein in the development of demyelinating diseases. Additionally, it could suggest the potential of CLIC1-antibody as a therapeutic target for multiple sclerosis.

Limitations

The study may have a limited number of participants, therefore, it is recommended for future studies to be done on a larger scale. Also, financial constraints restricted the detection of antibodies that target intracellular epitopes, which are not predicted to have a pathogenic effect. One possible explanation for the higher occurrence of seropositivity detected by ELISA compared to immunocytochemistry. Hence, employing immunoprecipitation and ELISA techniques that target the extracellular domain of CLIC1. This dual approach allows for both qualitative and quantitative assessments and could offer a more accurate and meaningful assessment of CLIC1 antibodies. Moreover, the lack of newly diagnosed patients and ethical limitations, such as the inability to withhold treatment, may have affected the study's outcomes In the current era of disease- modifying therapies (DMT) for MS, it has become challenging to evaluate antibody responses, as treatments like Fingolimod and Rituximab can impair humoral immunity and potentially affect the reliability of findings.

Conclusion

Anti-CLIC1 is considered a fair diagnostic biomarker for diagnosis of multiple sclerosis, however, it may not have a correlation with EDSS score and disease severity.

Supplementary Materials: None.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Additional data and materials may be available on request. Re-quests should be directed to the corresponding author.

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References

1. Ford ,H. Clinical presentation and diagnosis of multiple sclerosis. Clin Med (Lond). 2020,20(4),380–3.
2. Hauser ,S.;L.; Cree, B.;A.;C. Treatment of multiple sclerosis: a review. Am J Med. 2020,133(12),1380–90.e2.
3. Liu, R.; Du ,S.; Zhao, L.; Jain ,S.; Sahay ,K.; Rizvanov ,A.; et al. Autoreactive lymphocytes in multiple sclerosis: Pathogenesis and treatment target. Front Immunol. 2022,13,996469.
4. Alsharkawi, I.; Almutairi, F.; Taha, N.; Behbehani, R.; Alroughani, RPresentation and out-come of early-onset multiple sclerosis in Kuwait. Journal of Neurological Sciences. 2019, 407, 116489. https://doi.org/10.1016/j.jns.2019.116489
5. Karaaslan ,Z.; Şengül-Yediel, B.;Yüceer-Korkmaz ,H.; Şanlı ,E.; Gezen-Ak ,D.; Dursun ,E.; et al. Chloride Intracellular Channel Protein-1 (Clic1) Antibody In Multiple Sclerosis Patients With Predominant Optic Nerve And Spinal Cord Involvement. Mult Scler Relat Disord. 2023,78,104940.
6. Thompson, A. J.; Banwell, B. L.; Barkhof, F.; Carroll, W. M.; Coetzee, T.; Comi, G.; Correale, J.; Fazekas, F.; Filippi, M.; Freedman, M. S.; Fujihara, K.; Galetta, S. L.; Hartung, H.P.;Kappos, L.;Lublin, F. D.; Marrie, R. A.; Miller, A. E.; Miller, D. H.; Montalban, X.; Weinshenker, B. G. Di-agnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology.2018, 17(2), 162-173. https://doi.org/10.1016/S1474-4422(17)30470-2
7. Chan, Y.H. Biostatistics 102: Quantitative Data – Parametric & Non-parametric Tests. Singapore Med J. 2003,44(8),391-396.
8. Mcginley ,M.P.; Goldschmidt ,C.H.; Rae-Grant, A .D. Diagnosis And Treatment Of Multiple Sclerosis: A Review. JAMA. 2021,325,765-779.
9. Wang, H.; Wang, X.; Xu ,L.; Lin, Y.; Zhang, J.; Cao, H. Low Expression Of Cdhr1 Is An Inde-pendent Unfavorable Prognostic Factor In Glioma. J Cancer. 2021,12,5193-5205.
10. Wallin ,M.T.; Culpepper, W.J.; Coffman, P.; Pulaski ,S.; Maloni, H.;Mahan, C.M.; et al. The Gulf War Era Multiple Sclerosis Cohort: Age And Incidence Rates By Race, Sex And Service. Brain. 2012,135,1778-1785.
11. Naseri ,A.; Nasiri ,E.; Sahraian ,M.A.; Daneshvar ,S.; Talebi ,M. Clinical Features of Late-Onset Multiple Sclerosis: a Systematic Review and Meta-analysis. Mult Scler Relat Disord. 2021,50:102816.
12. Nguyen, H.; Lauto ,A.; Shanu ,A.; Myers, S.J. Chloride intracellular channel protein 1 and its role in neurodegenerative disorders and cancerous tumors. J Biochem Anal Biochem. 2016,5(1),249.
13. Karaaslan, Z.; Şengül-Yediel, B.; Yüceer-Korkmaz, H.; Şanlı, E.; Gezen-Ak ,D.;Dursun ,E.; et al. Chloride Intracellular Channel Protein-1 (Clic1) Antibody In Multiple Sclerosis Patients With Predominant Optic Nerve And Spinal Cord Involvement. Mult Scler Relat Disord. 2023,78,104940.
14. Fonzo, M.; Bertoncello, C.; Trevisan, A. Factors influencing long- term persistence of anti-HBs after hepatitis B vaccination. NPJ vaccines. 2022, 7(1), 173.