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Oligoclonal band versus chitinase-3-like protein-1 in CSF of newly diagnosed relapsing remitting multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune-mediated demyelinating disease of the central nervous system (CNS) that is usually associated with varying degrees of progressive disability. Chitinase-3-like protein-1 (CHI3L1) has attracted growing attention as a marker of ongoing inflammation and oncogenic transformation. The aim of this work was to assess the diagnostic accuracy of CHI3L1 versus IgG oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) of newly diagnosed relapsing remitting MS (RRMS) patients to throw light on a new simpler non subjective potential diagnostic marker in MS. This cross-sectional study of MS patients was carried at Ain Shams University Hospitals during the period from January 2021 till January 2022. Subjects included in this study were 40 patients diagnosed as having RRMS, based on their magnetic resonance imaging (MRI) findings, clinical presentation and according to the revised McDonald criteria 2017. The group included 10 males and 30 females; their ages ranged from 20 to 45 years. We found a significant correlation between CSF CHI3L1 levels and presence of oligoclonal bands (p=0.001), and that a cut off value of 30 ng/ml could be used for diagnosis of MS with sensitivity 84.85% and specificity 85.71%. A significant association was also found between CHI3L1 levels in CSF and Expanded Disability Status Scale (EDSS) score (p=0.002). We concluded that there were high levels of CHI3L1 in the CSF of MS patients and there was a significant correlation between CHI3L1 and oligoclonal bands and that CHI3L1 may be considered a promising diagnostic marker of MS.

Keywords: Oligoclonal Band, Chitinase-3-like Protein-1, CSF, Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune-mediated demyelinating disease of the central nervous system (CNS) that is usually associated with varying degrees of progressive disability. In most patients the early stages of the disease, known as relapsing remitting MS (RRMS) are characterized by clinical exacerbations, or relapses, caused by autoreactive immune cells that traffic into the CNS, resulting in focal inflammation and demyelination often visible as gadolinium-

enhancing lesions on magnetic resonance imaging (MRI). Relapses are followed by periods of clinical remission as inflammation resolves and remyelination occurs.[1]

Investigation of cerebrospinal fluid (CSF) in the diagnostic work-up of suspected MS patients has regained attention in the latest version of the diagnostic criteria due to its good diagnostic accuracy and increasing issues with misdiagnosis of MS based on over interpretation of neuroimaging results. The hallmark of MS-specific changes in CSF is the detection of oligoclonal bands (OCBs) which occur in the vast majority of MS patients. The laboratory methods for detection of CNS immunoglobulin synthesis are immunoglobulin G (IgG) index and gel isoelectric focusing with visual detection of OCBs, of which OCBs is considered the gold standard.[2] OCB positivity requires a minimum of two unique IgG bands in CSF, which are not present in serum. Both methods, however, have weaknesses. The relevance of IgG index in MS diagnostics has previously been questioned due to low sensitivity, OCBs have been reported in other primary and secondary CNS immune-mediated disorders (CIMD) that may clinically mimic MS such as CNS lupus, various forms of CNS vasculitis, neurosarcoidosis, antiphospholipid syndrome, CNS infections, CNS lymphoma and neuromyelitis optica spectrum disorder (NMOSD). In addition, OCB is time consuming, expensive, merely qualitative and due to its visual interpretation, it is prone to inconsistent results.[3] Therefore, the further search for other biomarkers which are less complicated and less subjective to detect is of great importance in order to improve the diagnosis and therapy of MS.[4]

Chitinase-3-like protein-1 (CHI3L1) has attracted growing attention as a marker of ongoing inflammation and oncogenic transformation. This secreted glycoprotein belongs to the 18-glycosyl-hydrolase family of proteins but lacks glycolytic activity. Although its biological functions are not fully understood, it is expressed by many cell types, including macrophages, neutrophils, chondrocytes, endothelial cells, microglia, and astrocytes.[5] In MS brain tissue, CHI3L1 is expressed in astrocytes in white matter plaques and in normal appearing white matter, and it is also expressed in microglia in MS lesions.[6] In addition, CHI3L1 mediate increased immune cell trafficking across the blood brain barrier. CHI3L1 is hypothesized to play a role in chronic inflammation and tissue remodeling.[7]

The present study aimed to assess the role of CSF CHI3L1 levels in the diagnosis of MS patients compared to OCBs in an attempt to throw light on a new simpler non subjective potential diagnostic marker in MS.

Patients and Methods

This was a cross-sectional study of MS patients attending the MS unit, Neurology Department at Ain Shams University Hospitals during the period from January 2021 till January 2022. Subjects included in this study were 40 patients diagnosed as having RRMS based on their MRI findings, clinical presentation and according to the revised McDonald criteria 2017.[8] The group included 10 males and 30 females, adult Egyptian patients with relapsing-remitting MS diagnosed according to the McDonald Criteria (2017); their ages ranged from 20 to 45 years. Patients having other neurological conditions affecting the CNS were excluded. Consent was obtained from the patients prior to enrolment in the study and they were informed about the aim; methodology and they had agreed to participate in the study.

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine, Ain Shams University (FMASU: MS 50/2021). A written informed consent was obtained from all the patients before participated in this study.

All patients were subjected to the following: full history taking, thorough neurological examination including initial assessment of MS according to the Revised McDonald criteria 2017 for MS diagnosis, MRI protocol for MS, and assessment of functional disability using Expanded Disability Status Scale (EDSS)[9] at the time of CSF sample withdrawal. EDSS score ranges from 1 to 10. A score from 1 to 4.5 refers to fully ambulatory MS patients while a score from 5 to 9.5 refers to MS patients with

impairment of ambulation and a score of 10 refers to death due to MS.

Two ml of CSF were collected from each patient included in this study under complete aseptic conditions. The CSF samples were stored at -20°C till used. OCBs in CSF samples were detected by isoelectric focusing with immunofixation using a capillary electrophoresis system (Sebia device provided by Parc Technologique Leonard de Vinci CP 8010 Lisses 91008 EVRY Cedex, France). A CSF sample with more than two OCB bands was considered positive. In addition, quantitative detection of CHI3L1 in CSF samples was performed using a commercially available ELISA kit (Bioassay Technology Laboratory (1088 Junjiang Inter, Bldg.228 Ningguo Rd. Yangpu Dist. Shanghai, China), according to the manufacturer's instructions. The laboratory work of this study was done for all patients in the Clinical Pathology Department, Ain Shams University.

Statistical analysis

The collected data were revised, coded, tabulated, and introduced to a PC using Statistical Package for Social Science (SPSS) (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0 Armonk, NY: IBM Corp). Suitable analysis of collected was done according to the type of data obtained for each parameter. The ROC curve analysis was performed to determine the sensitivity, specificity of CSF CHI3L1 for diagnosis of MS patients. The probability of error at 0.05 was considered significant.

Results

In the present study, a significant association was found between age and CHI3L1 levels (p=0.004). The sociodemographic, clinical, and laboratory data for all patients included in the present study are shown in (Table 1).

In the present study there was a significant association between CHI3L1 level and presence of OCB in CSF (p=0.001). (Table 2)

Table 1. The sociodemographic, clinical and laboratory data for all 40 patients included in the study.

		CSF OCBs					CSF CHI3L1	
		Negative Mean ± SD N (%)	Positive Mean ± SD N (%)	*P*-value			Median (IQR)	*p*-value
Age	≤ 30	4(57.14%)	13(39.39%)	NS	Age	≤ 30	30 (15 - 45)	0.004
	> 30	3(42.86%)	20(60.61%)			45 (45-120)	83.74 ± 69.28	
Sex	Male	3(42.86%)	7 (21.21%)	NS	Sex	Male	45 (15 - 60)	NS
	Female	4(57.14%)	26 (78.79%)			Female	45 (30 - 81)	
Smoking	No	5(71.43%)	27 (81.82%)	NS	Smoking	No	45 (37.5 - 60)	NS
	Yes	2(28.57%)	6 (18.18%)			Yes	45 (12 - 82.5)	
Family history of MS	Negative	5(71.43%)	31 (93.94%)	NS	Family history of MS	Negative	45 (45 - 60)	NS

*Significance was done for CSF oligoclonal bands (OCBs) by student t-test and for CSF Chitinase-3-like protein-1 (CHI3L1) by Mann-Whitney test. * p > 0.05 is not significant (NS). *Oligoclonal bands: Negative group included patients with ≤ 2 CSF bands while positive group included patient with > 2 CSF bands.[10]

Table 2. Association between OCB and CHI3L1 in the 40 CSF of patients studied.

		Chitinase 3 like 1 protein in CSF	Mann-Whitney test
		Median (25^{th} – 75^{th})	*p*-value
CSF oligoclonal bands	Negative	30 (15 - 30)	0.001
	Positive	45 (45 - 81)	

*$p \leq 0.05$ is significant.

A significant association was also found between CHI3L1 levels in CSF and EDSS score (p=0.002). However, no significant association was found between CHI3L1 levels in CSF and number of attacks or number of affected areas (p=0.201, p=0.322 respectively) (Table 3).

Table 3. Association between CHI3L1 and EDSS, number of attacks and number of affected areas in the patients studied.

		CH3LI CSF level (ng/ml)		*p* value
		Mean ± SD	Median (IQR)	
No. of attacks	1	47.31 ± 32.18	45 (30 - 45)	NS
	2	71.74 ± 71.75	45 (30 - 60)	
	3	100 ± 34.64	120 (60 - 120)	
No of affected areas	1	71.59 ± 62.91	45 (30 - 120)	NS
	2	61.5 ± 61.68	45 (30 - 60)	
	3	55 ± 8.66	60 (45 - 60)	
EDSS		r=0.465		0.002

*Significance for attacks and affected areas were done by Kruskal Wallis test and for EDSS score was done by Spearman's rho. $p > 0.05$ is not significant (NS).

No significant correlation was found between OCB and EDSS scores (p=0.460). Also no significant association was found between OCB and number of attacks or number of affected areas (p=0.859, p=0.449 respectively) (Table 4).

Table 4. Association between OCB and EDSS, number of attacks and number of affected areas in the 40 patients studied.

		CSF oligoclonal bands		*p* value
		Negative N (%)	Positive N (%)	
Numbers of attacks	1	3 (42.86%)	10 (30.3%)	NS
	2	4 (57.14%)	19 (57.58%)	
	3	0 (0%)	3 (9.09%)	
	4	0 (0%)	1(3.03%)	
Number of areas affected	1	5 (71.43%)	17 (51.52%)	NS
	2	1 (14.29%)	13 (39.39%)	
	3	1 (14.29%)	2 (6.06%)	
	4	0 (0%)	1 (3.03%)	
EDSS score				NS

*Significance done by student t-test. $p > 0.05$ is not significant (NS)

Using ROC curve analysis, a cut-off value of CHI3L1 in CSF >30 ng/ml was found to have a diagnostic sensitivity of 84.85%, specificity of 85.71% (Figure 1 & Table 5).

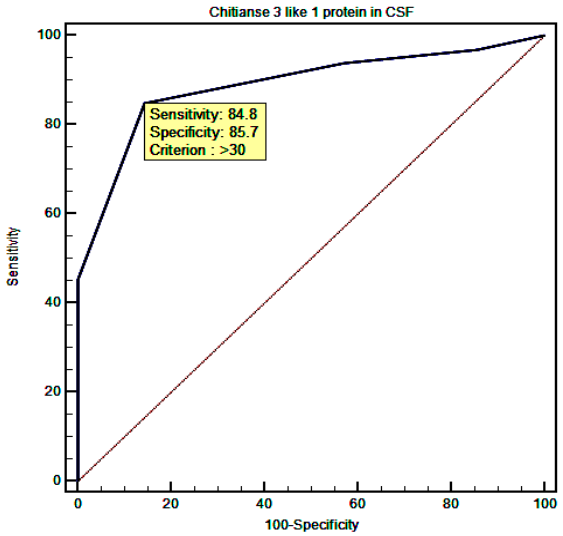

Figure 1. ROC of CHI3L1 in the studied patients.

Table 5. The diagnostic characteristics of CHI3L1 in the 40 studied patients.

AUC	95% CI	Sig.	Cut-off value	Sensitivity	Specificity	+LR	-LR	+PV	-PV
0.89	0.750 -0.966	<0.001	> 30	84.85	85.71	5.94	0.18	96.6	54.5

Discussion

The present study intended to assess the role of CSF CHI3L1 levels in the diagnosis of MS patients compared to OCBs to determine whether it can be used as a potential and simple non subjective diagnostic marker in MS.

In this study, MS patients were between 20 and 45 years of age with a mean of 31.98 years, among them 16 patients were below the age of 30 years and 24 patients were above the age of 30 years. A significant correlation was found between CSF CHI3L1 levels and the ages of patients (p=0.004). This finding is consistent with that of a study done by Kušnierová et al., (2020)[11] and Shneider et al., (2021).[12]

In this study, 25% of patients included were males, while females accounted for 75% of the MS studied patients. Our results showed no significant difference in the CHI3L1 CSF levels or OCBs with sex (p=0.37, p=0.338 respectively), smoking state (p=0.29, p=0.611, respectively) or family history of MS (p=0.257, p=0.134, respectively) among the studied MS patients. This finding is consistent with data reported in studies done by Correale and Fiol et al., (2011),[7] Hinsinger et al., (2015)[6] and Shneider et al., (2021).[12]

Determination of CSF CHI3L1 levels by ELISA is easy and reproducible compared to the interpretation of immunoelectrophoresis for the determination of OCB.[13] In this study, we found a significant association between CHI3L1 CSF levels and presence of OCB (p=0.001). On the contrary, Kušnierová et al., (2020),[11] found no significant association between CSF CHI3L1 levels and presence of oligoclonal banding. This discrepancy may be due to small sample sizes in both studies, subjectivity of visual interpretation of the OCB results, and different clinical stages of the studied MS patients in both studies.

A significant positive correlation was also found between CHI3L1 CSF level in MS patients and EDSS score. These results agreed with those of Pierez-Miralleo et al., (2020),[14] who reported that high CHI3L1 level was correlated with EDSS score at the beginning of the study and 12 months later during follow up. Similarly, Comabella et al., (2010),[15] Canto et al., (2015)[16] and Gil-Perotin et al., (2019),[17] reported a significant correlation between high CSF CHI3L1 levels with disease progression and development of disability.

Moreover, Comabella et al., (2010),[15] point to the possible use of CHI3L1 as a marker that could distinguish between clinically isolated syndrome (CIS) patients who will remain as CIS and those who will clinically convert to overt MS.

In the present study, no significant association was found between CSF CHI3L1 levels and number of affected areas in MRI of MS patients (p=0.322). There was also no significant association between CSF CHI3L1 levels and number of attacks (p=0.201), though a gradual increase in mean levels of CHI3L1 levels was noticed among patients with 1, 2 and 3 attacks which may reflect cumulative damage after repeated attacks. On the contrary, Comabella et al., (2010),[15] reported a significant association between numbers of affected areas and CSF CHI3L1. The discrepancy in the results could be due to small sample size and difference in patient selection.

In this study, we found that a cut off value of CHI3L1 in CSF at 30 ng/ml yielded sensitivity 84.85% and specificity 85.71% for the diagnosis of MS. Similarly, Metwally et al., (2021),[18] reported that a CH3L1 level of 133 ng/ml in CSF can discriminate demyelinating CNS diseases mainly MS from other non-demyelinating neurologic disorders.

We acknowledge that the sample size in this study was relatively small, and findings should be validated in larger patient populations. Collectively, our data together with earlier reports still strengthens the finding that there were high levels of CHI3L1 in CSF of MS patients.

In conclusion, our findings of high levels of CHI3L1 in the CSF of MS patients, the significant association between CHI3L1 and oligoclonal bands, and the positive correlation between CHI3L1 levels and EDSS score led us to conclude that CHI3L1 could be used as a potential valuable marker of disease progression and development of disability in MS patients.

Author Contributions

HT and ME, Conceptualization, methodology and study design. MEL, Resources, and data collection. LS, Data analysis and interpretation, writing- original draft, reviewing and editing. HG and DS, Investigation, project visualization, reviewing and editing. The authors have read and approved the manuscript.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University (FMASU MS 59/ 2021).

Informed consent

A written informed consent was taken from all the patients before participation in this study.

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