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RESEARCH ARTICLES

Cerebrospinal fluid HOXB3 does not differentiate relapsing–remitting multiple sclerosis from idiopathic intracranial hypertension: a pilot study

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ABSTRACT

Aim: Multiple sclerosis (MS) is a complex disease in terms of diagnosis and differential diagnosis due to the variety of clinical and radiological findings. Many diseases have similar characteristics and mimic MS. Thus, potential differential biomarkers have an importance for diagnosis of MS. Homeobox protein-3 (HOXB3) is a homeobox transcription factor implicated in immune cell regulation and neurodevelopment, and recent cerebrospinal fluid proteomic studies have suggested its potential role in neuroinflammatory processes, making it a plausible candidate biomarker for differential diagnosis in MS. The aim of this study was to investigate whether cerebrospinal fluid levels of HOXB3 differ between treatment-naïve patients with relapsing–remitting multiple sclerosis (RRMS) during relapse and patients with idiopathic intracranial hypertension (IIH).

Methods: Forty-one pwMS diagnosed with McDonald criteria were enrolled for the case groups. Thirty-four patients with IIH diagnosed with Dandy criteria were enrolled for control group. We measured cerebrospinal fluid level of HOXB3 (CSF HOXB3) by Enzyme-Linked Immunosorbent Assay (ELISA) method.

Results: No significant difference was observed in CSF HOXB3 levels between pwMS and control group. The ROC curve for HOXB3 was not statistically significant with the AUC at 0.573.

Conclusion: HOXB3 does not differentiate RRMS from IIH under the studied conditions.

Keywords: multiple sclerosis, human homeobox protein-3, cerebrospinal fluid, biomarker

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INTRODUCTION

Multiple sclerosis (MS) is a chronic and inflammatory disease that affects the central nervous system (CNS). The main characteristics of the disease are axonal loss and neuronal damage caused by the degeneration and demyelination of neurons (1). This disease is known to cause various symptoms that can affect the autonomic, motor, visual, and sensory systems. The underlying causes of the disease remain not yet fully understood, however, the incidence and prevalence of MS are increasing worldwide (2). MS is an inherently complex disease, and various disorders can easily mimic its clinical symptoms and paraclinical findings. This makes the diagnosis of MS particularly challenging due to the variability in clinical features and the lack of specific tests. Currently, there are no exclusive markers for diagnosing MS. Magnetic Resonance Imaging (MRI) is an essential tool in this diagnostic process. Although white matter lesions on brain MRIs are widely recognized as a hallmark of MS, they are also prevalent in other CNS inflammatory diseases, leading to potential diagnostic confusion. The growing availability of effective treatments for MS, coupled with the significant advantages of early intervention, underscores the critical need for accurate and timely diagnosis. Over the past decade, the landscape of cerebrospinal fluid (CSF) and blood biomarkers has significantly evolved, emerging as crucial tools for the diagnosis, prognosis, and treatment monitoring of MS (3).

Homeobox (HOX) genes are a family of homeodomain-containing transcription factors mainly involved in development (4). HOX genes regulate cell shape, migration, motility, proliferation and programmed cell death as well as conferring a differentiated cell identity. HOX genes are more highly expressed in haematopoietic stem cells, endothelial cells during vascular remodelling, endometrial cells of the uterus, fibroblast cells and skeletal stem cells, compared to less frequently dividing cells (5,6). Some HOX family members can cause inflammation in various disease states. As a result of these studies, HOX transcription factors may affect the inflammatory process and may be modulated by inflammation in many pathological conditions (7). Based on proteomic and ELISA studies, it has recently been reported that CSF levels of HOXB3 may serve to monitor the conversion from clinically isolated syndrome (CIS), the initial form of MS, to MS (8,9). Although the function of HOXB3 in the CNS is not fully understood, its growth regulatory effect and high expression in lymphocytes and leukaemia cells suggest that HOXB3 may be a potential marker of immune cell activation (4,10,11).

Considering the role of HOXB3 in the pathology of MS, in this pilot study, we aimed to investigate whether HOXB3 may confer a function as a differential biomarker in a cohort of pwMS during relapse period.

MATERIAL AND METHODS

Subjects and study procedure

41 newly diagnosed patients fulfilling the criteria for MS (12) were enrolled. Since CSF samples could not be obtained from healthy controls due to the disapproval of the ethics committee, 34 patients fulfilling the criteria for idiopathic intracranial hypertension (IIH) (13) were included as control group. Patients with other autoimmune diseases and other coexisting neurological or systemic disorders were excluded to avoid cross-reactivity. The study protocol was approved by Bolu Abant İzzet Baysal University Local Ethical Committee under decision number 2024/29 on March 3rd, 2024, and followed the guidelines outlined in the Helsinki Declaration. All the subjects gave written informed consent. CSF samples were collected from all participants during the first clinical episode, and patients were not under any immunosuppressive or immunomodulating treatment All the patients underwent the Lumbar Puncture (LP) for CSF sampling. The samples were divided into aliquots and stored at -80°C until assay.

Study of CSF samples

The human ELISA kit (Sunred Biological Technology Co., Ltd in Shanghai, China) was used for HOXB3 assay. The sensitivity is 0.033 ng/mL. Before testing, all the CSF samples were carefully thawed at 2-8°C and allowed to reach room temperature for 30 minutes. The assay was performed according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed by using GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA, USA). Frequency and percentage were used for categorical-demographic data of the groups. The Kolmogorov-Smirnov test was used to determine the distributional characteristics of continuous variables. In descriptive statistics, mean ± standard deviation values were provided for normally distributed characteristics, while median (minimum-maximum) values were given for non-normally distributed characteristics. Comparisons between two independent groups were assessed through the Student T-test and Mann–Whitney test, as appropriate. The linear association between the variables was examined through the Pearson correlation coefficient. Receiver Operating Characteristic (ROC) curve was fitted to estimate the ability of CSF HOXB3 concentration in distinguishing groups. The best cut-off value for this variable was determined with the Youden test. The statistical significance level was accepted as $p < 0.05$.

RESULTS

Demographic and clinical features of the study cohort

The relapsing-remitting MS (RRMS) subtype was selected for the pwMS. The primary IIH subtype was chosen for the IIH. Upon analyzing the gender distribution of participants, 87.8% of the pwMS were female and 12.2% were male. In the IIH group, 82.4% of the patients were female and 17.6% were male. The average age for the pwMS and IIH was 32.4 and 41.4, respectively. The age of onset was lower in pwMS than IIH. The median value for EDSS was 2.00 for pwMS. The average IgG index was higher in pwMS than IIH. Table 1 shows the demographic and clinical characteristics of the groups.

Quantification of CSF HOXB3 level

The mean CSF HOXB3 concentrations for pwMS and IIH were 3.42 ± 0.81 and 3.63 ± 0.81, respectively. No statistically significant difference was observed in CSF HOXB3 level between the groups (p=0.267) (Figure 1A). Acording to the gender; CSF HOXB3 level was lower in men than women (p=0.037) (Figure 1B).

Relationship between CSF HOXB3 and disability

The median EDSS score was 2.00 (1.0–2.5) for pwMS, and no significant correlation was found between CSF HOXB3 and EDSS score (r= 0.1594, p= 0.368).

ROC curve for CSF HOXB3

The 95% confidence interval of the ROC curve of pwMS and IIH was from 0.4420 to 0.7029. The area under the curve, AUC, was 0.5725 (p=0.282). The best cut-off value was 2.475. As per this cut-off point, the sensitivity and specificity of HOXB3 were 0.9706 and 0.1463, respectively (Figure 2).

Table 1. Demographic and clinical characteristics of the study cohorts

Characteristics		RRMS (n=41)	IIH (n=34)	P value
Age, years [a]		32.4±9.1	41.4±11.9	**<0.001**
Gender [b]	Male	5 (12.2)	6 (17.6)	0.506
	Female	36 (87.8)	28 (82.4)	
IgG Index [c]		0.87 (0.13-4.32)	0.51 (0.08-6.81)	**<0.001**
OCB [b]	Negative	8 (20.0)	32 (97.0)	**<0.001**
	Positive	33 (80.0)	1 (3.0)	

RRMS: Relapsing-Remitting Multiple Sclerosis, IIH: Idiopathic Intracranial Hypertension, EDSS: Expanded Disability Status Scale, IgG: Immunoglobulin G, OCB: Oligoclonal band.
[a]: Mean ± standard deviations. P value was determined via the Independent Samples T-test for age.
[b]: Values are frequency (%). P values were determined via the Chi-Square test.
[c]: Values are medians (min. – max.). P value was determined via the Mann-Whitney U test.
Bold p values indicate statistical significance.
The assessment for OCB was not conducted in 1 patient with IIH. IgG index data was made up of 40 patients with MS and 24 patients with IIH.

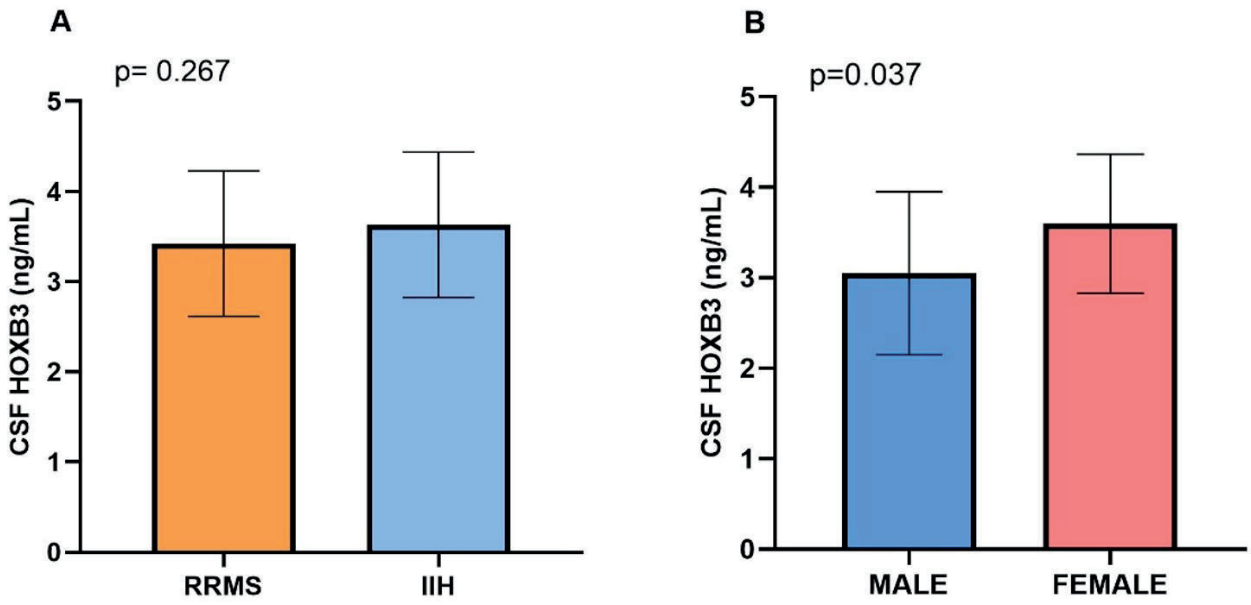

Figure 1. Comparison of CSF HOXB3 levels (A, B). (A) No difference was found in CSF HOXB3 levels between pwMS and IIH. (B) CSF HOXB3 levels were higher in female compared with male. The error bar indicates mean ± standard deviation. Comparisons between two independent groups were assessed through the Student T-test.

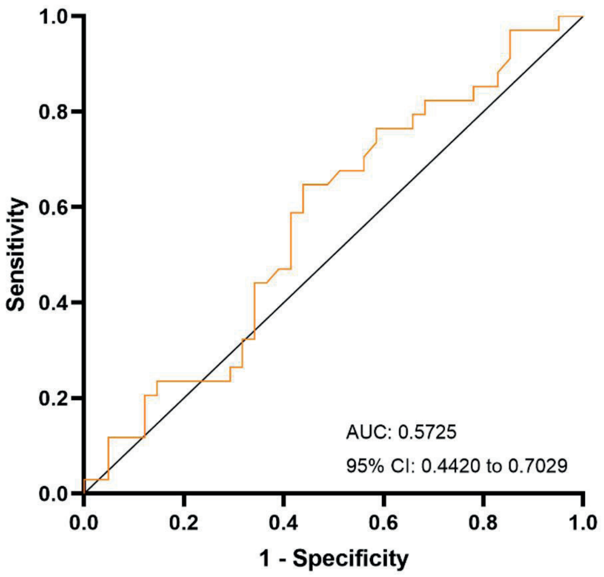

Figure 2. ROC curve of CSF HOXB3 concentration. The 95% confidence interval is from 0.4420 to 0.7029. The area under the ROC curve is 0.5725.

DISCUSSION

In this pilot study, we measured HOXB3 level in CSF obtained during the first clinical attack within the first 30 days of the relapse. We found no significant difference in CSF HOXB3 level in pwMS than IIH which is another infilamatory disease. Although HOXB3 is recognised as a transcription factor involved in the embryonal stages of neurogenesis and brain development (14), its role in autoimmunity is largely unknown. Our results indicated that CSF HOXB3 levels in pwMS were not statistically different from those of the control group under the present study conditions. Previous reports have demonstrated that biomarker measurements may vary depending on whether sampling is performed during relapse or remission (8,9,15). Therefore, our findings suggest that CSF HOXB3 does not differentiate pwMS from controls during the relapse phase in this cohort. At the same time, this observation illustrates a broader challenge in MS biomarker research, namely that reliance on a single candidate molecule may not provide adequate diagnostic or prognostic performance. Increasing evidence supports the use of multimodal biomarker panels capable of reflecting the complex and dynamic nature of MS. In this context, integrative strategies combining proteomic, metabolomic, transcriptomic, and epigenetic data may facilitate the identification of more robust molecular signatures for disease characterization, relapse prediction, and treatment monitoring.

An intriguing finding was that the CSF HOXB3 level in IIH was similar with pwMS. IIH is a rare disease in which intracranial pressure is increased in the absence of intracranial pathology and normal cerebrospinal fluid composition (16). The two most prominent symptoms of IIH are papillary oedema and progressive visual impairment resulting from chronic headache. Additional symptoms such as cranial nerve palsies, cognitive disorders, tinnitus and olfactory dysfunction are frequently part of the clinical picture (17). Since CSF sample from healthy individuals can not obtained due to ethical procedures, samples of patients with IIH are generally used a control group in studies (18). However, the accuracy of using IIH as a control group has begun to be discussed in recent studies due to the similar characterization of the some conditions present in IIH such as pericyte degeneration, blood-brain barrier dysfunction, and perivascular aquaporine-4 expression as in chronic degenerative diseases (19-22). This challenges the validity of IIH as a true control group in biomarker studies and underscores the need for alternative disease controls or statistical approaches that adjust for overlapping biological pathways.

In the levels of some biomarkers, variation according to gender is likely to be a situation, however, no study showing the relation of HOXB3 level with gender difference was found in literature. In our study, we observed higher CSF HOXB3 level in female than male. Although they are preliminary, our finding of sex-related differences in HOXB3 levels corroborate the neccessity of specific analyses based on sex and hormone in biomarker research. Sex hormones are increasingly recognized as modulators of immune and neural processes in multiple sclerosis (23,24), and sex-based analyzes may uncover slight yet clinically significant biomarker patterns.

Several limitations are present in this study. We included samples obtained during the relapse period, because of this, our results should be interpreted within the context of acute disease activity. Since samples obtained during the remission period were not analyzed, potential differences in biomarker levels between relapse and remission could not be determined. Longitudinal studies across various disease periods are necessary to clarify potential temporal variations.

The approximately 10-year age difference between the MS and IIH groups causes an important limitation of this study. Age-related biological variability may have impacted the measured biomarker levels independent of disease status. Therefore, age should be considered a potential confounding effect when interpreting the results, and the observed differences should not be attributed only to disease mechanisms.

The relatively small sample size is an another limitation of the study. This situation is related to the limited number of patients presenting to the neurology clinic and the difficulties associated with enrolling newly diagnosed, drug-naïve individuals. As a result, statistical power of the study may be reduced and the generalizability of the findings may be limited. Clinical studies in MS patients are typically conducted in individuals who have already received treatment, and this may causes certain biomarkers undetectable and reduce the reliability of the results (25). Although the relatively small sample size is a limitation, the recruitment of newly diagnosed, drug-naïve MS patients increases the value of the study. CSF samples obtained from drug-naïve individuals provides a more accurate assessment of HOXB3 levels, free from potential confounding effects of prior treatment, and gives a different insight into the molecular profile at the earliest stage of the disease. Longitudinal sampling across both relapse and remission phases with larger cohorts, combined with imaging and clinical assessments in future studies will be critical for fully understanding the temporal dynamics of candidate molecules such as HOXB3.

CONCLUSION

This pilot study provides the methodological baseline for exploring biomarker in MS. Beyond HOXB3 as a biomarker, the data increase the importance of multi-marker strategies, standardized sampling across disease phases, rigorous control group definition, and the consideration of sex-specific effects.

However, because idiopathic intracranial hypertension (IIH) implied the control group, the interpretability of HOXB3 as a differential biomarker is naturally limited. Within the concept of the present study, HOXB3 did not find a differential parameter for RRMS. Accordingly, these findings cannot be generalized for differential diagnosis of MS.

Future investigations incorporating diverse and disease-relevant control populations will be necessary to determine the true diagnostic utility of HOXB3.

Ethical approval

This study has been approved by the Bolu Abant İzzet Baysal University Local Ethical Committee (approval date 03/03/2024, number 2024/29). Written informed consent was obtained from the participants.

Author contribution

Concept: FU; Design: FU; Data Collection or Processing: FU; Analysis or Interpretation: FU; Literature Search: FU; Writing: FU, FB, NH, SAT. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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