Clinica Chimica Acta 476 (2018) 49–53

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Elevated serum levels of endothelin-1 in patients with chronic inflammatory demyelinating polyneuropathy

Chun-Wei Chang[a], Hsiu-Chuan Wu[a,b], Rong-Kuo Lyu[a,b], Yen-Shi Lo[a,b], Chiung-Mei Chen[a,b], Long-Sun Ro[a,b], Hong-Shiu Chang[a,b], Ching-Chang Huang[a,b], Ming-Feng Liao[a,b], Yih-Ru Wu[a,b], Hung-Chou Kuo[a,b], Chun-Che Chu[a,b], Yi-Ching Weng[a,b], Pei-Tsi Wei[a,b], Ai-Lun Lo[b], Kuo-Hsuan Chang[a,b,*]

[a] Department of Neurology, Chang Gung Memorial Hospital-Linkou Medical Center, No.5, Fusing St., Gueishan Dist., Taoyuan City 333, Taiwan
[b] Chang Gung University College of Medicine, Taoyuan, Taiwan, No.261, Wenhua 1st Rd., Guishan Dist., Taoyuan City 333, Taiwan

ARTICLE INFO

Keywords:
Chronic inflammatory demyelinating polyneuropathy
Endothelin-1
Biomarker

ABSTRACT

Introduction: Chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired, or non-hereditary, chronic demyelinating neuropathy. Currently, there is no reliable molecular biomarker that can identify CIDP patients as well as monitor disease severity.

Material and methods: We measured serum levels of endothelin-1 (ET-1), a factors involved in vasoconstrictive, inflammatory and nerve regenerative processes, in 20 CIDP, 21 acute inflammatory demyelinating polyneuropathy (AIDP), 37 multiple sclerosis (MS), and 10 Alzheimer's disease (AD) patients, as well as 26 healthy control (HC) subjects.

Results: Patients with CIDP demonstrated higher serum levels of ET-1 (2.07 ± 1.07 pg/mL) than those with AIDP (0.75 ± 0.62 ng/mL, $P < 0.001$), AD (0.78 ± 0.49 pg/mL, $P < 0.001$), as well as HCs (1.16 ± 0.63 pg/mL, $P = 0.002$), while levels of ET-1 in patients with MS (2.10 ± 0.81 pg/mL) and CIDP were similar. Furthermore, the serum ET-1 levels significantly correlated with Inflammatory Neuropathy Cause And Treatment (INCAT) disability scale in CIDP patients. Receiver operating characteristic (ROC) curve showed good discrimination ability for ET-1 to distinguish CIDP patients from AIDP (AUC = 0.883) or HCs (AUC = 0.763).

Conclusion: This study discloses the potential of serum ET-1 as a biomarker for CIDP.

1. Introduction

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an acquired chronic immune-mediated inflammatory peripheral neuropathy [1]. It is typically characterized by a relapsing-remitting or progressive course of weakness with areflexia and distal large fiber sensory loss [1]. Nerve conduction studies show multi-focal demyelination and cerebrospinal fluid (CSF) protein content is elevated with a normal CSF leukocyte count [1]. Although these clinical features and diagnostic tests are straightforward, the classical presentations are frequently absent particularly in the acute relapsing phase of patients with CIDP [2–4]. Thus a biomarker is urgently needed to ensure robust diagnosis for CIDP.

The exact mechanisms that underlie the development of CIDP have not been fully understood. Lines of evidence support that CIDP is an autoimmune disease mediated by humoral and/or cellular immunity against undefined Schwann cell/myelin antigens [5]. Sural nerve biopsies show prominent infiltrating inflammatory cells including CD8+ T cells, CD4+ T cells and macrophages in CIDP patients [6,7]. Proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-2, are highly expressed within the injured nerves [8]. Although these pathological findings raise the expectation to apply proinflammatory cytokines as biomarkers for CIDP, the attempts to measure the alternation of these molecules in peripheral tissues still get disappointing results [9,10], and the reliable biomarker for CIDP is still missing.

Endothelin-1 (ET-1) is a potent vasoconstrictor widely distributed in the body, including the eye [11], neurons and glia cells [12]. ET-1 activates inflammatory transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and up-regulates expressions of proinflammatory cytokines including TNF-α, IL-1 and IL-6 [13]. These transcription factors and proinflammatory

* Corresponding author at: Department of Neurology, Chang Gung Memorial Hospital-Linkou Medical Center, No.5, Fusing St., Gueishan Dist., Taoyuan City 333, Taiwan.
E-mail address: gophy5128@cgmh.org.tw (K.-H. Chang).

https://doi.org/10.1016/j.cca.2017.11.008
Received 11 July 2017; Accepted 11 November 2017
Available online 11 November 2017
0009-8981/

Table 1
Clinical characteristics of the patients with CIDP, AIDP, MS, AD and healthy control.

Parameter	CIDP (N = 20)	AIDP (n = 21)	MS (n = 37)	AD (n = 10)	HC (n = 26)
Gender (male/female)	11/9	11/10	12/25	5/5	11/15
Age (years)	49.3 ± 14.63	43.10 ± 18.06	34.19 ± 13.32	75.70 ± 6.24[a]	45.00 ± 15.73
Hugh's functional grading scale		2.88 ± 1.39			
INCAT disability scale	3.90 ± 2.76				
EDSS			3.15 ± 2.31		
IgG index	0.61 ± 0.13	0.66 ± 0.13	1.25 ± 0.52#		
Endothelin-1 (pg/mL)	2.07 ± 1.07[b]	0.75 ± 0.62	2.10 ± 0.81[b]	0.78 ± 0.49	1.16 ± 0.63

INCAT disability scale: Inflammatory Neuropathy Cause And Treatment (INCAT) disability scale; EDSS: Kurtzke Expanded Disability Status Scale; CIDP: Chronic inflammatory demyelinating polyneuropathy; AIDP: Acute inflammatory demyelinating polyneuropathy; MS: Multiple sclerosis; AD: Alzheimer's disease; HC: Healthy control.
[a] : Statistically significant in comparison with AIDP, CIDP, MS and HC.
[b] : Statistically significant in comparison with AIDP, AD and HC.

cytokines in turn stimulate ET-1 production [14]. Given that the aberrant immune response also contribute to the pathogenesis of CIDP, ET-1 is likely to play a role in this peripheral inflammatory neuropathy. In the present study, we measured serum levels of ET-1 in patients with CIDP, acute inflammatory demyelinating polyneuropathy (AIDP) and multiple sclerosis (MS); these levels were also compared with those of healthy controls (HCs) and patients with Alzheimer's disease (AD).

2. Material and methods

2.1. Ethics approval and consent to participate

Permissions to obtain venous blood from patients and HCs were obtained from the Institutional Review Boards of the Chang Gung Memorial Hospital (ethical license No: 103-4555C).

2.2. Sample collection

Serum samples were collected from 20 CIDP (9 females, 11 males), 21 AIDP (10 females, 11 males), 37 MS (25 females, 12 males), and 10 AD (5 females, 5 males) patients, as well as 26 HCs (15 females, 11 males) in Chang Gung Memorial Hospital in Taiwan. The diagnosis of CIDP [15], AIDP [16], MS [17] and AD [18] were made according to respective diagnostic criteria. The neurological disability of the patients at the time of venepuncture was assessed using Inflammatory Neuropathy Cause And Treatment (INCAT) disability scale (for patients with CIDP) [19], Hughes' functional grading scale (for patients with AIDP) [20], or Kurtzke Extended Disability Status Scale (EDSS, for patients with MS) [21] on the basis of the results of neurological examination and ambulatory ability assessment. All subjects were confirmed to have no systemic infection, chronic renal failure, cardiac or liver dysfunction, malignancies, or autoimmune diseases other than CIDP, AIDP, MS and AD. In patients with AIDP and MS, serum and CSF samples were collected within two weeks after the onset or acute relapses of diseases. All samples were obtained before treatment with corticosteroid, plasmapheresis, or intravenous immunoglobulins. Sample analyses were blindly performed with respect to patients' diagnosis and the results of other tests from the same patient. Serum samples were kept sitting at 4 °C for 4 h and then centrifuged, aliquoted, frozen at − 80 °C, and stored until analysis. The serum and CSF albumin and IgG levels were measured by the Department of Clinical Pathology, Chang Gung Memorial Hospital.

2.3. Enzyme-linked immunosorbent assays for quantification of ET-1 expression in serum

Serum levels of ET-1 were assessed using enzyme-linked immunosorbent assay (ELISA) Kit (R & D). Each assay was measured in duplicate for each sample at the same time.

2.4. Statistical analysis

The Statistical Program for Social Sciences (SPSS) was used to analyze all the statistics. Non-categorical variables were compared using Kruskal-Wallis test (non-parametric test to compare unmatched groups by multiple comparison analysis). Spearman correlation were applied to evaluate the relationship between serum ET-1 level and INCAT disability scale, Hughes' functional grading scale or EDSS at the time of sample collection. Each set of data was expressed as mean ± standard deviation. All P values were 2-tailed, and a $P < 0.05$ was considered significant. An analysis of the receiver operating characteristic (ROC) curve was used to measure the ability of serum ET-1 level to distinguish CIDP patients from the AIDP patients or HCs.

3. Results

The demographic data of all groups were displayed in Table 1. The age of patients with AD (75.70 ± 6.24 years) was significantly higher than the other groups ($P < 0.001$). Immunoglobulin G (IgG) index in patients with MS (1.25 ± 0.52) was significantly higher compared with those with CIDP (0.61 ± 0.13, $P = 0.002$) and AIDP (0.66 ± 0.13, $P = 0.001$). Patients with CIDP showed higher serum levels of ET-1 (2.07 ± 1.07 pg/mL) than those with AIDP (0.75 ± 0.62 ng/mL, $P < 0.001$) and AD (0.78 ± 0.49 pg/mL, $P < 0.001$), as well as HCs (1.16 ± 0.63 pg/mL, $P = 0.002$). Serum levels of ET-1 in patients with MS (2.10 ± 0.81 pg/mL) were similar to those with CIDP. We further analyzed the correlation between the serum levels of ET-1 and disease severity that was evaluated by scores of INCAT disability scale (for the patient with CIDP), Hughes' functional grading scale (for the patients with AIDP), or EDSS (for the patients with MS) at the time point of sample collection. The results showed a significant correlation between serum levels of ET-1 and INCAT disability scale in patients with CIDP ($P = 0.005$, Fig. 1A). Serum levels of ET-1 were also significantly correlated with EDSS in patients with MS ($P = 0.001$, Fig. 1C). Nevertheless, such clinical correlation is absent in patients with AIDP (Fig. 1B). With the estimated standard deviations in each marker, at the level of 0.05, present sample sizes achieved a power of 99.89% for ET-1 to detect differences in the mean between CIDP and AIDP. The power of ET-1 to detect differences in the mean between CIDP and HCs was 94.24%. Area under the ROC curve (AUC) showed good discrimination ability for ET-1 to distinguish CIDP patients from AIDP (AUC = 0.883) (Fig. 2A) or HCs (AUC = 0.763) (Fig. 2B). The CSF level of ET-1 was undetectable by the assay we used.

4. Discussion

Currently, there is no reliable biomarker for CIDP. In the present study, we demonstrated that serum ET-1 level is higher in CIDP patients when compared with AIDP and AD patients, as well as HCs. Importantly, our data showed a positive correlation between serum