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Evaluation of CD137 and CD137L Transcript Levels and the Serum sCD137 in Immune-mediated Polyneuropathy

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ABSTRACT

Background: Abnormal humoral and cellular immune responses have been reported in immune-mediated polyneuropathies. CD137, as a costimulatory molecule and a TNF receptor superfamily member, has been demonstrated to have a key role in the pathogenesis of many autoimmune as well as inflammatory disorders.

Objective: To evaluate the transcripts levels of CD137, its ligand (CD137L), and the serum levels of soluble CD137 (sCD137) in patients with immune-mediated polyneuropathy.

Methods: A total of 45 patients and 46 sex and age-matched healthy individuals were enrolled in the study. CD137 and CD137L transcript levels were assessed by the Real-Time PCR, and the serum level of sCD137 was measured using the ELISA technique. The Bayesian regression model was used for statistical analysis at the 0.05 significance level in R 4.1.0 statistical environment.

Results: Transcript levels of the CD137 and CD137L were higher in polyneuropathy patients in comparison with the healthy subjects (P=0.006 for both). Conversely, the mean level of sCD137 was significantly lower in the sera of patients compared to the controls (P<0.001).

Conclusion: Our findings point to the possible role of CD137 and CD137L in immune-mediated polyneuropathy pathogenesis. More investigations are required to clarify the exact contributions of the mentioned molecules to the pathogenesis of immune-mediated polyneuropathies.

Keywords: CD137 Antigen, Chronic Inflammatory Demyelinating Polyradiculoneuropathy, Tumor Necrosis Factor Receptor, Superfamily, sCD137

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INTRODUCTION

Immune response to auto-antigens in the peripheral nervous system (PNS) can cause immune-mediated polyneuropathies which can be acute such as the Guillain-Barre syndrome (GBS) or chronic as in the case of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). As a Th1-mediated autoimmune disease, the GBS includes several subtypes among which acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is the most common one. Peripheral neuropathies are generally characterized by progressive weakness of muscles. [1-3]. It is worth mentioning that the most common cause of acute flaccid paralysis is the GBS with an approximate incidence of 1-2/100,000 per year [4]. It seems that the GBS and CIDP are different variants of the same disorder in which the acute state is considered as the GBS, while CIDP with slow and chronic symptoms lies at the other end of the clinical spectrum [5].

CD137 or 4-1BB is a tumor necrosis factor receptor (TNFR) family member. It can transmit both costimulatory and death signals. Membrane-bound CD137 cross-linking can trigger apoptosis in activated T cells in a Fas-independent pathway. As reported, soluble forms of some receptors have been found in the TNFR family, among which the soluble CD137 (sCD137) has been elucidated to have a critical role in the regulation of inflammation in autoimmune disorders through negative feedback. The level of sCD137 has been shown to inversely correlate with lymphocyte proliferation [6]. In autoimmune patients, altered serum sCD137 levels have been reported by several previous studies [6, 7]. CD137 is produced in various cells such as B cells, T cells, natural killer (NK) cells, mast cells, and eosinophils [8]. According to previous reports, sCD137 could disrupt the interaction of CD137 with its ligand. The major source of sCD137 is the regulatory T cell (Treg cell), and altered numbers of $CD4^+$ $FoxP3^+$ $CD25^+$ Treg cells have been observed in the acute phase of the GBS [9, 10]. CD137 Ligand (CD137L) is produced and expressed on the surface of antigen-presenting cells (APCs). Under physiological circumstances, CD137-CD137L interaction can promote the survival and proliferation of CD137-expressing cells [11]. Growing evidence suggests the involvement of CD137 and CD137L in the pathogenesis of some autoimmune diseases [12]. Moreover, previous reports have shown that the immune system plays a critical role in immune-mediated polyneuropathy development and progression. For instance, some studies have demonstrated an altered expression of costimulatory molecules including B7 and inducible costimulator (ICOS) in the GBS and CIDP patients [13, 14].

Although no study has investigated CD137 expression in immune-mediated polyneuropathies thus far, there are some reports on other Th1-mediated disorders [7]. It has been shown that CD137 stimulation by agonistic antibody or natural ligand can reduce the severity of systemic lupus erythematosus (SLE), experimental autoimmune encephalomyelitis (EAE), inflammatory bowel disease (IBD) and collagen-induced arthritis (CIA) in mouse models [15].

Even though CD137 and CD137L have been suggested to act as key role players in the context of autoimmunity, their accurate contribution has not been fully elucidated. Due to the lack of knowledge on the alterations of CD137 and CD137L in polyneuropathies, in this study, we sought to explore CD137 and CD137L expression levels as well as the serum sCD137 in patients with immune-mediated neuropathy.

MATERIALS AND METHODS

Study Groups

In the current case-control study, blood samples were collected from 45 immune-

mediated polyneuropathy patients. CIDP and AIDP patients were recruited from Sina hospital and Imam Khomeini Clinic, Hamadan, Iran within 2019-2020. Moreover, 46 sex and age-matched healthy subjects were considered as the control group. The patients were diagnosed as per the criteria provided by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the American Academy of Neurology [16, 17]. Patients with cancer, recent infection, or any systemic disease were excluded. This study was conducted according to the Institutional and/or National Research Committee Ethical standards, the 1964 Helsinki declaration and its later modifications or comparable ethical standards. The study was confirmed by the Ethical Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.082). All the participants signed informed consent forms. The methods followed all the relevant regulations and guidelines.

ELISA

Peripheral blood (five mL) was obtained in gel tubes and subsequently centrifuged. The serum was kept at −80 °C till use. The level of sCD137 was assayed using a Human sCD137 ELISA kit 96T (ZellBio, Germany) based on biotin double-antibody sandwich technology. Briefly, 10 µl of sCD137-Ab and 50 µl streptavidin-HRP were added to 40 µl of serum samples. After incubation at 37 °C for 60 min, washing was performed with the provided wash buffer. Chromogen solution (100 µl) was added before incubating for 15 min at 37 °C. Finally, after adding the Stop solution, the OD values were recorded at 450 nm.

Gene Expression Analysis

Peripheral blood samples, in five mL volumes, were obtained and the total RNA was isolated using a Hybrid-RTM blood total RNA extraction kit (GeneAll, Seoul, South Korea). The quantity and quality of RNA were assessed by NanoDrop One^{c} equipment (Thermo Scientific, MA, USA) and gel electrophoresis respectively. OneStep RT-PCR Series Kit (BioFact™, Seoul, South Korea) was utilized for the complementary DNA (cDNA) synthesis.

PCR program involved an activation phase at 95 °C for 15 min, 45 cycles of 95 °C for 15 seconds, 57 °C (GAPDH and CD137L) or 60 °C (CD137) for 30 seconds, and 72 °C for 30 seconds. The reaction volumes comprised of 20 µL [containing 2 µL cDNA, 10 µl Master Mix, 4 µL double distilled deionized water, and 2 µL of each primer (Metabion, Germany). The values of the cycle threshold (Ct) were corrected for amplification efficiency. The efficiency values for CD137, CD137L, and GAPDH were 1.9, 1.8, and 1.98, respectively. The formula: Efficiency$^{-\Delta CT}$ was employed to calculate the relative gene expression. The expression of CD137 and CD137L was evaluated by Q1000^{+} Real-Time PCR System (LongGene, China) with RealQ Plus 2x PCR Master Mix Green Without ROX™ (Ampliqon, Odense, Denmark). Table 1 represents the details of the used primers. The experiments were performed in duplicate.

Statistical Analysis

To hold the normality assumption of the relative expression of CD137 and CD137L

Table 1. Characteristics of the primers used in this study

Gene	Primer sequence	Primer and Probe length	Product size	Accession number
CD137	F: ACGGGGCAGAAAGAAACTCC	20	97 bp	NM_001561.6
	R: TGGAAATCGGCAGCTACAGCCA	22		
CD137L	F: GGCCTGAGCTACAAAGAGGA	20	129 bp	NM_003811.4
	R: CAGCGCAAGTGAAACGGAG	19		
GAPDH	F:CATCAAGAAGGTGGTGAAGCAG	22	120 bp	NM_001357943
	R: GCGTCAAAGGTGGAGGAGTG	20		

CD137L: CD137 ligand; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

genes in the case and the control groups, the $\log_{10}$ (Efficiency$^{-\Delta CT}$) was applied for the subsequent analysis. To describe the relative expression of the genes in each group according to gender, boxplots were presented.

The Bayesian regression model and Gaussian prior family were utilized to compare the mean values of the relative expression of CD137 and CD137L genes between the case and the control groups. Using this statistical method, the effects of age, gender, and their interactions were evaluated. To predict the disease status, the receiver operating characteristic (ROC) regression model was used to evaluate optimal cut-off points for the gene expression. This model was used to calculate the specificity (Sp), sensitivity (Se), and the area under the ROC curve (AUC), as well as the optimal cut-off points based on the Youden index J (JI). Monte Carlo chains were used with 6000 iterations and 1000 warm-up steps to evaluate the parameters of the Bayesian regression model and calculate the P-values and the 95% credible interval (95% CrI). Moreover, the associations between the age and relative expression levels of the genes were assessed using a correlation matrix. All the analyses were applied at the 0.05 significance level using ggplot2, rstanarm, pROC, bayestestR, and psych packages in the R software (version 4.1.0).

RESULTS

Demographic Data of Participants

A total of 45 patients participated in our study. The control group included 46 healthy individuals who were sex- and age-matched with cases. Table 2 represents the demographic data of the subjects.

Gene Expression of CD137, CD137L and the Serum Levels of sCD137

Transcript levels of CD137 and CD137L were significantly higher in patients compared to the healthy subjects (Posterior Beta=-0.52 and -0.66 respectively, P=0.006 for both) with the former being overexpressed in the male subjects in comparison with the female ones (Posterior Beta=0.52, P=0.031). While both genes were significantly upregulated in the male patients compared to the male controls (Posterior Beta=-0.67, P=0.003 and Posterior Beta=-0.72, P=0.009 for CD137 and CD137L, respectively), the expression levels of these genes in the female case and the control subjects were not significantly different (P=0.946, P=0.373 for CD137 and CD137L, respectively). Moreover, we found that the serum sCD137 level in the patient group was significantly lower than that in the control group (Posterior Beta=242, P<0.001). Similar significant differences were also observed in both the female and male subgroups of the two groups, separately (Posterior Beta=217.99, P<0.001; Posterior Beta=228.57, P=0.014 for the males and females, respectively). Furthermore, the sCD137 level was significantly lower in the male gender compared to the female one (Posterior Beta=-134.24, P=0.023). As for the age, although significant reverse associations were found with CD137L gene expression and the serum sCD137 (Posterior Beta=-0.02, P=0.035; Posterior Beta=-3.88 P=0.012,

Table 2. Demographic data of the studied groups as number or the mean±SD

Variables	Control	Patients
Female/male (no. (%))	10(21.7%)/36(78.3%)	11(24.4%)/34(75.6%)
Age (mean±SD, year)	51.74±14.09	51.64±17.42
Age range (year)	19-86	18-82
Duration of the disease (years)	-	4.8±3.2
CIDP/AIDP	-	29/16

CIDP: Chronic inflammatory demyelinating polyradiculoneuropathy; AIDP: Acute inflammatory demyelinating polyradiculoneuropathy

respectively), there was not any considerable association with CD137 expression. Detailed data have been shown in Table 3 and Figure 1.

ROC Curve Analysis

Based on AUC values, optimal cutoff points for CD137, CD137L, and sCD137 were exponential modes of 0.04, -3.61, and 1336.93, respectively. Furthermore, the Se values for CD137, CD137L, and sCD137 were 65.22%, 37.5%, and 67.44%, respectively. Also, the Sp values for the same order of the genes were 68.18, 95%, and 87.80, respectively (Figure 2). Comparisons of AUC, Se, and Sp values

Table 3. Relative expression of CD137 and CD137L and the serum levels of sCD137 in the patients and the controls

Variable		logCD137				logCD137L				sCD137			
		Posterior Beta	SE	95% CrI	P value	Posterior Beta	SE	95% CrI	P value	Posterior Beta	SE	95% CrI	P value
Total	Group (Control/ Case)	-0.52	0.20	[-0.91, -0.15]	0.006	-0.66	0.23	[-1.11, -0.21]	0.006	242.00	48.88	[144.75, 338.05]	<0.001
	Gender (Male/ Female)	0.52	0.24	[0.05, 0.98]	0.031	-0.07	0.28	[-0.61, 0.49	0.813	-134.24	59.15	[-249.58, -19.24]	0.023
	Age	-5.64e-04	6.20e-03	[-0.01, 0.01]	0.924	-0.02	7.19e-03	[-0.03, 0.00]	0.035	-3.88	1.54	[-6.88, -0.87]	0.012
Male	Group (Control/ Case)	-0.67	0.22	[-1.10, -0.24]	0.003	-0.72	0.27	[-1.25, -0.19]	0.009	217.99	58.79	[101.51, 331.09]	<0.001
	Age	-2.57e-03	6.53e-03	[-0.02, 0.01]	0.683	-0.01	8.10e-03	[-0.03, 0.00]	0.082	-3.87	1.74	[-7.29, -0.40]	0.028
Female	Group (Control/ Case)	-0.03	0.52	[-1.08, 1.00]	0.946	-0.39	0.46	[-1.29, 0.52]	0.373	228.57	83.53	[-9.35, 10.60]	0.014
	Age	8.20e-03	0.02	[-0.03, 0.04]	0.655	-0.02	0.02	[-0.05, 0.01]	0.123	-2.76	2.90	[-8.33, 3.06]	0.340

sCD137: Soluble CD137

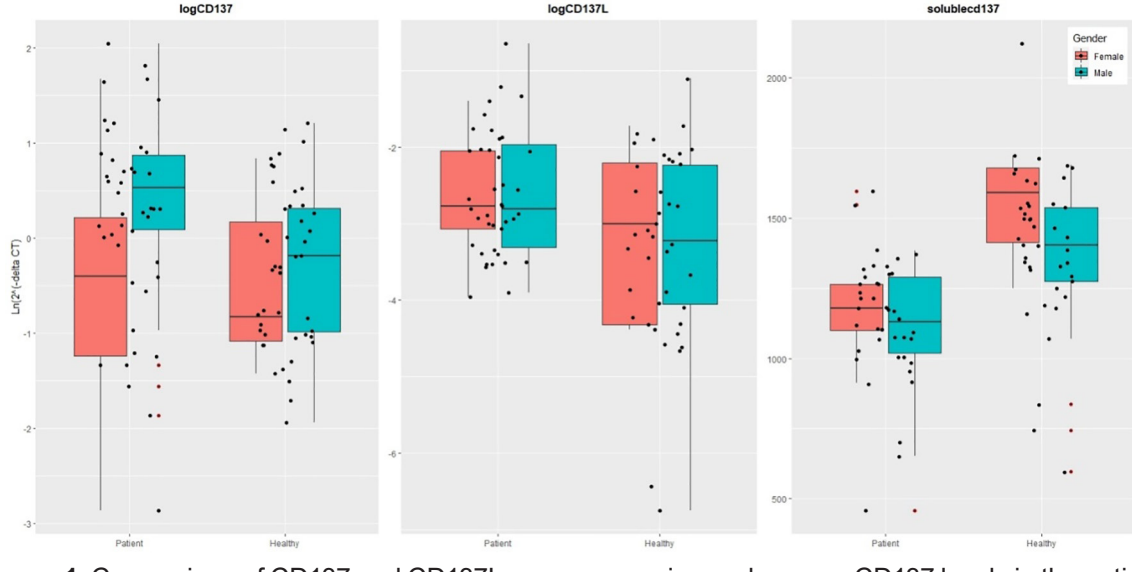

Figure 1. Comparison of CD137 and CD137L gene expression and serum sCD137 levels in the patients and the controls based on the gender of study participants. *P value<0.05, ** P value<0.01, ***P value<0.001

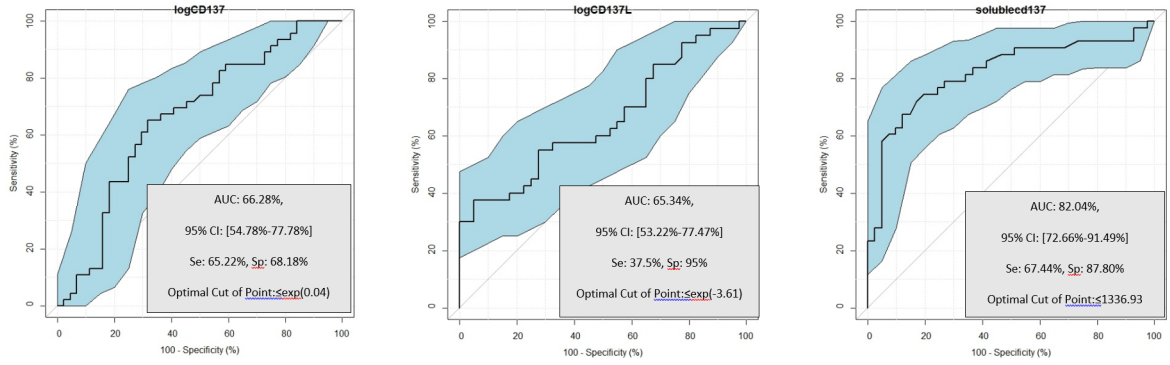

Figure 2. Results of ROC curve analysis for CD137, CD137L and sCD137. Results from Bayesian regression model. AUC: Area under curve, CI: Confidence interval, Se: Sensitivity, SP=Specificity.

among the three molecules suggest that sCD137, besides other markers, might serve as a useful tool in the diagnostic panels of polyneuropathies

Correlation Analysis

A significant inverse correlation was found between the age and the sCD137 and also between the age and CD137L transcript levels (r=-0.24, r=-0.23, respectively, P<0.05 for both). In addition, the serum sCD137 inversely correlated with CD137 transcription level (r=-0.28, P<0.01). As we know, in the correlation matrix, the correlation coefficients between -1-0 and 0-1 show negative and positive statistical correlations between the studied parameters, respectively. Details of correlation analysis can be seen in Figure 3.

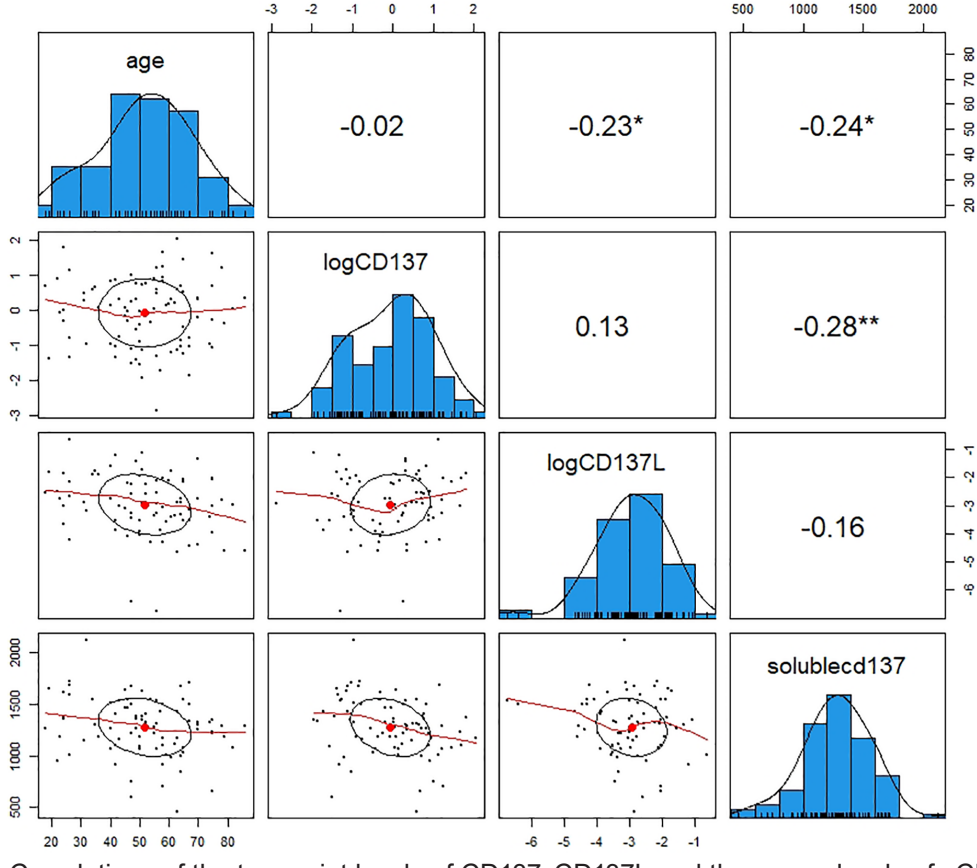

Figure 3. Correlations of the transcript levels of CD137, CD137L and the serum levels of sCD137 with age and with each other. There were significant inverse correlations between the age and the sCD137 and also between the age and CD137L transcript levels. Furthermore, the serum sCD137 level was inversely correlated with CD137 transcription levels. **P value=0.001-0.01, *P value<0.05

DISCUSSION

CD137/CD137L interaction plays a costimulatory role in various T cell subsets causing different immunological responses [18]. The sCD137 is the soluble form of CD137 produced via alternative splicing [7]. Although some studies have been conducted on CD137 and sCD137 in some Th1-mediated autoimmune disorders, these molecules have not been studied in immune-mediated polyneuropathies, hitherto. We found that there was a significant decrease in the serum sCD137 in immune-mediated polyneuropathy patients compared to the control group. Itoh et al. in 2019 and Lushnikova et al. in 2021 reported that the serum sCD137 significantly decreased in pediatric type 1 diabetes and lymphocytic colitis patients, respectively [7, 19]. These observations bring to mind the notion that in Th1-mediated autoimmune disorders, regardless of the disease origin, the serum sCD137 levels considerably decreased. Alterations in the sCD137 levels appear to be important as this molecule can interfere with CD137/CD137L interaction thereby mitigating T cell activation. Since Treg cells are deemed as a major source of the sCD137, and also based on the evidence regarding an altered number of $CD4^+$ $CD25^+$ $FoxP3^+$ Treg cells in the acute phase of GBS, changes in the sCD137 levels were predictable [9, 10]. Conversely, some studies have reported elevated sCD137 levels in Th1-mediated autoimmune diseases like rheumatoid arthritis and relapsing-remitting multiple sclerosis [6, 20]. Several factors are suggested to account for these contradictions including disease stage, sampling conditions, people's genetic differences as well as the presence of an accompanying disease.

Our results also showed increased transcript levels of CD137 and CD137L in polyneuropathy patients compared to healthy subjects. As expected, the results of the correlation analysis and the increasing or decreasing trends of these molecules in pairs, confirmed the results of statistical analysis on the relative gene expression and serum levels.

In another part of our study, by the Bayesian regression model and Gaussian prior family, the mean values of relative expressions of the genes between the study groups were compared and the effects of age, gender, and their interactions were analyzed. Using this model, we found that the CD137 gene expression is higher in males compared to the females. In addition, while the expression levels of CD137 and CD137L were higher in the male patients in comparison with the male controls, we found no considerable differences in the transcript levels of these genes between the female subjects of the two groups. According to this statistical model, the serum sCD137 levels showed a considerable decrease in the males compared to the females regardless of the group (case and control). Based on this finding, gender appears to have an important role in the sCD137 expression. We also found that the sCD137 levels in patients of both genders significantly decreased compared to the respective healthy controls. Considering the age in total participants, although there were significant reverse associations between age and CD137L expression and the serum sCD137 levels, we found no considerable association between the age and CD137 expression.

Based on ROC curve analysis and due to the high diagnostic power of the sCD137 (for the prediction and diagnosis of patients from the healthy controls) and acceptable Se and Sp values, the sCD137 could be employed as a useful marker, besides other markers, in diagnostic panels of polyneuropathies.

Interestingly, Yoshimori et al. in 2014 showed that Epstein Barr Virus (EBV) can induce in-vitro CD137 expression [21]. It is worth mentioning that EBV infection is possibly associated with GBS [22, 23]. On such a basis, EBV-induced CD137 expression might contribute to the development of GBS in EBV-infected people. The occurrence of GBS following some infections may be related to the increased expression of CD137. Moreover, some asymptomatic infections are also associated with this syndrome [22, 24,

25], highlighting the issue that the lack of a clinically-diagnosed infection history does not necessarily indicate that the patient is infection-free.

As mentioned, GBS has been known as a Th1-mediated polyneuropathy and in this regard, the percentage of Th1 cells has been found to increase in CIDP patients' cerebrospinal fluid (CSF) compared to other non-inflammatory neurological diseases [3, 26]. Interestingly, CD137/CD137L interaction is one of the major drivers of Th1 responses [9, 27]. In addition, the bidirectional signaling of CD137 and its ligand has a critical role in the pathogenesis of some autoimmune disorders. Julia M. Martínez Gomez et al. in 2012 reported that CD137Lknockout mice in the EAE exhibited decreased neuroinflammation [28, 29]. In addition, it has been elucidated that CD137 stimulation can lead to M1-like features in monocytes/macrophages. M1 macrophages are known to be associated with inflammation-mediated impairment of the myelin sheath by promoting Th1-mediated cytokines production and cellular cytotoxicity in the early stages of GBS [30, 31].

Consistent with our findings, the CD137 transcription level has been shown to be elevated in some other Th1-mediated autoimmune diseases (e.g. in biopsy of Crohn's disease-affected tissues) [32]. In this line, the study of Hyo Won Jung et al. on rheumatoid arthritis patients showed an inverse relationship between sCD137 and membrane-bound CD137 [6]. Hence, decreased serum sCD137 levels and increased mRNA levels of CD137 in our study could be interpreted as elevated levels of membrane-bound CD137. Since agonistic anti-CD137 antibodies have been shown to enhance the population of Treg cells, thereby inhibiting or alleviating some murine models of autoimmune diseases e.g. type 1 diabetes mellitus, [28] CIA and uveoretinitis [27], CD137 mediated manipulation of immune cells in the desired way (based on the disease type) seems to be beneficial. This probable therapeutic approach requires supporting evidence and more detailed future investigations.

CONCLUSION

In conclusion, while CD137 and CD137L, as costimulatory markers, showed significantly increased expression levels in peripheral blood cells of immune-mediated polyneuropathy patients in comparison with the healthy controls, the serum sCD137 level was decreased in these patients in comparison with the control group. Besides, the age and gender type were shown to affect the expression of these molecules. Future studies are warranted to delineate the precise effects of these molecules and the related signaling pathways in immune-mediated polyneuropathies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATION

This study was conducted following the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethical Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1400.082). Informed consent forms were obtained from all participants. All methods were performed following the relevant guidelines and regulations.

AVAILABILITY OF DATA AND MATERIALS

The analyzed data sets generated during the study are available from the corresponding author in case of reasonable request.

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AUTHORS' CONTRIBUTION

ST performed the experiments. ETG analyzed the data. MME and AZ supervised the study. MM was the clinical consultant. GR helped as a scientific advisor. All authors approved the manuscript.

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Conflict of Interest: None declared.

REFERENCES

1. Kieseier BC, Mathey EK, Sommer C, Hartung HP. Immune-mediated neuropathies. Nature reviews Disease primers. 2018;4(1):31. https://doi.org/10.1038/s41572-018-0027-2
2. Dimachkie MM, Barohn RJ. Guillain-Barré syndrome and variants. Neurol Clin. 2013;31(2):491-510. https://doi.org/10.1016/j.ncl.2013.01.005
3. Li S, Jin T, Zhang H-L, Yu H, Meng F, Concha Quezada H, et al. Circulating Th17, Th22, and Th1 Cells Are Elevated in the Guillain-Barré Syndrome and Downregulated by IVIg Treatments. Mediators of inflammation. 2014;2014:740947. https://doi.org/10.1155/2014/740947
4. Leonhard SE, Mandarakas MR, Gondim FAA, Bateman K, Ferreira MLB, Cornblath DR, et al. Diagnosis and management of Guillain–Barré syndrome in ten steps. Nature Reviews Neurology. 2019;15(11):671-83. https://doi.org/10.1038/s41582-019-0250-9
5. van Doorn PA. Treatment of Guillain-Barré syndrome and CIDP. Journal of the peripheral nervous system : JPNS. 2005;10(2):113-27. https://doi.org/10.1111/j.1085-9489.2005.0010203.x
6. Jung HW, Choi SW, Choi JI, Kwon BS. Serum concentrations of soluble 4-1BB and 4-1BB ligand correlated with the disease severity in rheumatoid arthritis. Exp Mol Med. 2004;36(1):13-22. https://doi.org/10.1038/emm.2004.2
7. Itoh A, Ortiz L, Kachapati K, Wu Y, Adams D, Bednar K, et al. Soluble CD137 Ameliorates Acute Type 1 Diabetes by Inducing T Cell Anergy. Frontiers in Immunology. 2019;10. https://doi.org/10.3389/fimmu.2019.02566
8. Ye L, Jia K, Wang L, Li W, Chen B, Liu Y, et al. CD137, an attractive candidate for the immunotherapy of lung cancer. Cancer science. 2020;111(5):1461-7. https://doi.org/10.1111%2Fcas.14354
9. Luu K, Shao Z, Schwarz H. The relevance of soluble CD137 in the regulation of immune responses and for immunotherapeutic intervention. J Leukoc Biol. 2020;107(5):731-8. https://doi.org/10.1002/JLB.2MR1119-224R
10. Liu S, Dong C, Ubogu EE. Immunotherapy of Guillain-Barré syndrome. Human vaccines & immunotherapeutics. 2018;14(11):2568-79. https://doi.org/10.1080/21645515.2018.1493415
11. Grimmig T, Gasser M, Moench R, Zhu LJ, Nawalaniec K, Callies S, et al. Expression of Tumor-mediated CD137 ligand in human colon cancer indicates dual signaling effects. Oncoimmunology. 2019;8(12):e1651622. https://doi.org/10.1080%2F2162402X.2019.1651622
12. Ajami M, Nazari M, Mahmoodzadeh H, Moazzeni SM. Recombinant CD137-Fc, its synthesis, and applications for improving the immune system functions, such as tumor immunotherapy and to reduce the inflammation due to the novel coronavirus. Journal of Cellular Biochemistry. 2021;122. https://doi.org/10.1002/jcb.29928
13. Kiefer R, Dangond F, Mueller M, Toyka KV, Hafler DA, Hartung HP. Enhanced B7 costimulatory molecule expression in inflammatory human sural nerve biopsies. Journal of neurology, neurosurgery, and psychiatry. 2000;69(3):362-8. https://doi.org/10.1136%2Fjnnp.69.3.362
14. Hu W, Janke A, Ortler S, Hartung HP, Leder C, Kieseier BC, et al. Expression of CD28-related costimulatory molecule and its ligand in inflammatory neuropathies. Neurology. 2007;68(4):277-82. https://doi.org/10.1212/01.wnl.0000250240.99311.9d
15. Zhang X, Voskens CJ, Sallin M, Maniar A, Montes CL, Zhang Y, et al. CD137 Promotes Proliferation and Survival of Human B Cells. The Journal of Immunology. 2010;184(2):787. https://doi.org/10.4049/jimmunol.0901619
16. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy

(CIDP). Neurology. 1991;41(5):617. https://doi.org/10.1212/WNL.41.5.617
17. Van der Meché FG, Van Doorn PA, Meulstee J, Jennekens FG. Diagnostic and classification criteria for the Guillain-Barré syndrome. Eur Neurol. 2001;45(3):133-9. https://doi.org/10.1159/000052111
18. Liu G-z, Gomes AC, Putheti P, Karrenbauer V, Kostulas K, Press R, et al. Increased Soluble 4-1BB Ligand (4-1BBL) Levels in Peripheral Blood of Patients with Multiple Sclerosis. Scandinavian Journal of Immunology. 2006;64(4):412-9. https://doi.org/10.1111/j.1365-3083.2006.01796.x
19. Lushnikova A, Bohr J, Wickbom A, Münch A, Sjöberg K, Hultgren O, et al. Patients With Microscopic Colitis Have Altered Levels of Inhibitory and Stimulatory Biomarkers in Colon Biopsies and Sera Compared to Non-inflamed Controls. Frontiers in medicine. 2021;8:727412. https://doi.org/10.3389%2Ffmed.2021.727412
20. Jafarinia M, Ashja-Arvan M, Hosseininasab F, Vakili S, Sadeghi E, Etemadifar M, et al. Evaluation of plasma soluble CD137 level in relapsing-remitting multiple sclerosis patients in comparison with healthy controls in Isfahan Province, Iran. Neurology Asia. 2020;25:361-5.
21. Yoshimori M, Imadome K-I, Komatsu H, Wang L, Saitoh Y, Yamaoka S, et al. CD137 Expression Is Induced by Epstein-Barr Virus Infection through LMP1 in T or NK Cells and Mediates Survival Promoting Signals. PLOS ONE. 2014;9(11):e112564. https://doi.org/10.1371/journal.pone.0112564
22. Grose C, Feorino P. EPSTEIN-BARR VIRUS AND GUILLAIN-BARRE SYNDROME. The Lancet. 1972;300(7790):1285-7. https://doi.org/10.1016/s0140-6736(72)92654-2
23. Tam CC, O'Brien SJ, Petersen I, Islam A, Hayward A, Rodrigues LC. Guillain-Barré Syndrome and Preceding Infection with Campylobacter, Influenza and Epstein-Barr Virus in the General Practice Research Database. PLOS ONE. 2007;2(4):e344. https://doi.org/10.1371/journal.pone.0000344
24. Umapathi T, Lim CS, Ooi EE, Zhang SL, Goh EJ, Tan HC, et al. Asymptomatic dengue infection may trigger Guillain-Barré syndrome. Journal of the peripheral nervous system : JPNS. 2016;21(4):375-7. https://doi.org/10.1111/jns.12190
25. Mokhashi N, Narla G, Marchionni C. Guillain-Barre Syndrome in a Patient With Asymptomatic Coronavirus Disease 2019 Infection and Major Depressive Disorder. Cureus. 2021;13(3):e14161. 10.7759/cureus.14161
26. Chi L, Xu W, Zhang Z, Huang H, Zhang L, Zhou J. Distribution of Th17 cells and Th1 cells in peripheral blood and cerebrospinal fluid in chronic inflammatory demyelinating polyradiculoneuropathy. Journal of the peripheral nervous system : JPNS. 2010;15:345-56. https://doi.org/10.1111/j.1529-8027.2010.00294.x
27. Dharmadhikari B, Wu M, Abdullah NS, Rajendran S, Ishak ND, Nickles E, et al. CD137 and CD137L signals are main drivers of type 1, cell-mediated immune responses. Oncoimmunology. 2016;5(4):e1113367. https://doi.org/10.1080/2162402x.2015.1113367
28. Wong HY, Schwarz H. CD137 / CD137 ligand signalling regulates the immune balance: A potential target for novel immunotherapy of autoimmune diseases. J Autoimmun. 2020;112:102499. https://doi.org/10.1016/j.jaut.2020.102499
29. Martínez Gómez JM, Croxford JL, Yeo KP, Angeli V, Schwarz H, Gasser S. Development of experimental autoimmune encephalomyelitis critically depends on CD137 ligand signaling. J Neurosci. 2012;32(50):18246-52. https://doi.org/10.1523/jneurosci.2473-12.2012
30. Stoll A, Bruns H, Fuchs M, Völkl S, Nimmerjahn F, Kunz M, et al. CD137 (4-1BB) stimulation leads to metabolic and functional reprogramming of human monocytes/macrophages enhancing their tumoricidal activity. Leukemia. 2021;35(12):3482-96. https://doi.org/10.1038/s41375-021-01287-1
31. Shen D, Chu F, Lang Y, Geng Y, Zheng X, Zhu J, et al. Beneficial or Harmful Role of Macrophages in Guillain-Barré Syndrome and Experimental Autoimmune Neuritis. Mediators of inflammation. 2018;2018:4286364. https://doi.org/10.1155/2018/4286364
32. Maerten P, Geboes K, De Hertogh G, Shen C, Cadot P, Bullens DMA, et al. Functional expression of 4-1BB (CD137) in the inflammatory tissue in Crohn's disease. Clinical immunology. 2004;112 3:239-46. https://doi.org/10.1016/j.clim.2004.04.009