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# Peripheral blood brain-derived neurotrophic factor level and tyrosine kinase B expression on T lymphocytes in systemic lupus erythematosus: Implications for systemic involvement

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ABSTRACT

*Objective:* Brain-derived neurotrophic factor (BDNF) has been reported to be involved in the pathogenesis of autoimmune diseases and tyrosine kinase B (TrkB) is the specific receptor for BDNF. Our aim in this study was to investigate serum BDNF level and TrkB expression on peripheral blood T cell surface in patients with systemic lupus erythematosus (SLE) and explore potential relationship between serum BDNF and SLE.

*Methods:* Samples from fifty SLE patients and thirty healthy controls were evaluated. Serum BDNF level was measured by enzyme-linked immunosorbent assay (ELISA) and the percentages of TrkB expression on the surface of CD3 + CD4 + and CD3 + CD8 + T lymphocytes were measured by flow cytometry. The SLE patients were divided into subgroups according to whether they exhibited brain, kidney or lung involvement, and whether the disease was active or inactive.

*Results:* Serum BDNF levels in SLE patients were decreased when compared to the controls ($p < 0.001$). Comparing with the SLE individuals without systemic involvement, the BDNF levels were decreased in SLE patients with lupus nephritis ($p = 0.042$) and in SLE patients with neuropsychiatric manifestations ($p = 0.04$). On the other hand, the BDNF level was significantly increased in the inactive SLE group ($p < 0.001$) compared to the active SLE group. In addition, the percentages of TrkB expression on CD3 + CD4 + and CD3 + CD8 + T cell surface in SLE were significantly higher ($p < 0.001$; $p < 0.001$, respectively) than that in the controls.

*Conclusions:* Serum BDNF level combined with TrkB expression on T cell surface can reflect SLE activity. It is possible that BDNF may be used as a potential serological biomarker for disease activity of SLE. In addition, the significant decrease in serum BDNF level may imply systemic involvement of SLE, as well as, possibly, differentiate neuropsychiatric SLE from hormone-induced mental disorders.

## 1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by an immune response against nuclear antigens, and deposition of immune complexes in target organs causing inflammation and tissue damage. Such targets of SLE include the nervous, renal and pulmonary systems [1]. Neuropsychiatric systemic lupus erythematosus (NPSLE) is one of the most serious complications of the disease [2–5] which leads to high mortality. On the other hand, lupus nephritis (LN) is considered as a common complication, as well as the most common cause of death in SLE patients besides infections [6]. Notwithstanding the health implications of the disease, the pathogenesis of SLE remains unclear.

Brain-derived neurotrophic factor (BDNF) plays important roles in promoting growth, differentiation and survival of neurons [7,8]. As such, BDNF has been demonstrated potential therapeutic value in Alzheimer's and Huntington's diseases [9–10]. In addition to the nervous system, BDNF has recently been shown to be present in peripheral blood. Lymphocytes are one of the main sources of BDNF [11] and they also express the specific receptors to BDNF, tyrosine kinase B (TrkB) [12–14]. BDNF can enhance proliferation of T lymphocytes and affect the differentiation of T lymphocytes into subsets [15]. Additionally, BDNF displays antiapoptotic effects in T lymphocytes [16–18]. For example, Garcia-Suarez [19] detected massive lymphocyte apoptosis in

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the thymus of functionally deficient TrkB mice and Maroder [20] showed an enhanced survival of isolated thymocyte precursors after BDNF application. Furthermore, Schuhmann [21] also proved that total thymocyte counts were significantly reduced in BDNF-deficient mice. Thus, BDNF is involved in inflammatory and autoimmune responses by acting on a wide variety of immune cells in regulating immune functions [22,23].

Serum BDNF has been studied in systemic sclerosis [24], primary Sjogren syndrome [25] and rheumatoid arthritis [26]. Similarly, local overexpression of both BDNF and TrkB have been reported in spondyloarthritis [27]. SLE is characterized as a loss of T-cell tolerance towards self-antigens in genetically predisposed individuals. Consequently, there is a large production of autoantibodies [28] and imbalance between Th17 and regulatory T-cells [29]. A few studies reported on serum BDNF level in SLE [30–33], but the results were inconsistent. The aim of our study, therefore, is to examine serum BDNF level and TrkB expression on the surface of peripheral blood T lymphocytes in SLE patients.

## 2. Materials and Methods

### 2.1. Patients and controls

Fifty consecutive individuals with SLE between Dec 2017 and Mar 2018 as well as thirty healthy controls in the same period were recruited from the Department of Rheumatology of the First Affiliated Hospital of China Medical University. All the SLE patients fulfilled the new international collaborating clinics classification criteria for SLE [34]. Disease activity was evaluated by the SLEDAI. SLE patients were divided into active (SLEDAI score ≥ 5) and inactive (SLEDAI score ≤ 4) groups [35,36]. Comorbidity with other autoimmune diseases, current or chronic infections, neoplastic disorders, endocrine and metabolic diseases and drug abuse were excluded. All the enrolled individuals had never taken any antidepressants.

All blood samples, both for patients and controls, were withdrawn at 8o'clock in the morning after an overnight fast. The blood samples were centrifuged to obtain serum after clot formation between 10:30–11:00, and serum samples were stored at −80 °C. Serum BDNF level was measured by Elisa within three months of sample collection. The anticoagulated blood samples were disposed with the lymphocyte separation medium (Ficoll-Paque™ PLUS, Amersham Biosciences) to get PBMCs within 2 h.

### 2.2. Ethical considerations

All study protocols were approved by the Ethics Committee of the First Affiliated Hospital of China Medical University (No. 2018-214-3). The study was conducted according to the principles expressed in the Declaration of Helsinki. Informed consents were obtained after the study was described and explained to all the participants.

### 2.3. Systemic involvement and laboratory parameters

All the SLE patients with neuropsychiatric manifestations were diagnosed following the 1999 ACR consensus [37]. Psychiatric diagnosis was performed by two trained psychiatrists and neurological diagnosis depended on cranial MRI, electroencephalogram and/or analysis of cerebrospinal fluid. All the NPSLE patients were assessed again by rheumatologists, psychiatrists and neurologists. ILD in SLE patients were diagnosed by two rheumatologists on the basis of high-resolution computed tomography (HRCT) and pulmonary function tests. Clinically persistent proteinuria > 0.5 g/24 h, repeatedly dysmorphia red cells ≥3/HP in urine or renal biopsy were used as diagnosis of LN. Blood lymphocyte counts, platelet counts (PLT), fibrinogen (Fib), D-dimer (D-D), immunoglobulin (IgG, IgA, IgM), complements (C3, C4) and T cell subsets were measured at the time of taking blood samples and were recorded for further analysis.

### 2.4. Measurement of serum BDNF level by ELISA

Total serum BDNF level was measured using commercial enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (DBNT00, R&D Systems, Minneapolis, USA). In order to minimize inter-samples variance resulting from experimental variation, all the serum samples were measured simultaneously. All assays were performed in duplicate and BDNF data was presented as pg/ml.

### 2.5. Detection of TrkB expression on the surface of CD3 + CD4+/ CD3 + CD8 + T cells by flow cytometry

The PBMCs were incubated with the appropriate antibody coupled to fluorescent dyes for 30 min at 4 °C. The stained samples were washed once and analyzed by FACSortk Flow cytometer and Cell Quest software (BD Biosciences), and the results were analyzed using the FlowJo v10 software (Tree Star, Ashland, OR, USA). The percentages of TrkB expression on CD3 + CD4 + and CD3 + CD8 + T cell surface were calculated after gating on CD3 + CD4 + or CD3 + CD8 + T lymphocytes. The stained antibodies were a combination of phycoerythrin (PE) -conjugated CD3 antibody (clone HIT3a; BD Biosciences); fluorescein isothiocyanate (FITC)-conjugated CD4 antibody (clone RPA-T4; BD Biosciences); phycoerythrin (PE)-cyanin (Cy)7-conjugated CD8 antibody (clone RPA-T8; BD Biosciences); Alexa Fluor®647-conjugated TrkB antibody (Mouse IgG1 Clone#72509, FAB3971R; R&D Systems) and isotype for TrkB (Mouse IgG1κClone#11711, IC002R; R&D Systems).

### 2.6. Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparison (Turkey post hoc test) were performed to compare the serum BDNF differences in groups of SLE patients with and without systemic involvement. The Student's *t*-test was used to examine the serum BDNF differences between SLE patients and controls. Pearson regression was performed to estimate the correlation between serum BDNF level and laboratory parameters. Analysis was performed using the SPSS 17.0 software and GraphPad Prism for Windows version 5.00 (Graph Pad Software, La Jolla, CA, USA) with two-tailed p values less than 0.05 considered significant.

## 3. Results

### 3.1. Participants characteristics

The mean age (standard deviation (SD)) of fifty SLE patients was 31.9 ± 14.9 years, age range (16–64 years old), and the average duration of disease was 3.5 years. Twenty-six naïve SLE patients were enrolled and the others were SLE patients suffering a relapse. The average SLEDAI score was nine. Complications observed in the SLE patients included: LN (25; 50%), ILD (8; 16%), and neuropsychiatric symptoms (6; 12%). Among the patients with neuropsychiatric symptoms, three had epilepsy, two suffered from stroke and one patient manifested psychosis. Additionally, thirty healthy controls, matched for age-and gender, were enrolled in the study.

The number of CD4 + T and CD3 + T cells in SLE was decreased when compared with the controls (296.3 ± 154.9/μl vs. 776.2 ± 172.6/μl, $p < 0.001$; 661.3 ± 344.4/μl vs. 1124.6 ± 469.6/μl, $p = 0.001$). Moreover, the number of CD8 + T cells in SLE was also lower than that in controls, however, the difference was not statistically significant (335.11 ± 205.72/μl vs. 362.30 ± 196.98/μl). In SLE individuals, the number of CD4+, CD8 + and CD3 + cells were negatively correlated with SLEDAI score

**Table 1**
Demographic and clinical characteristics of individuals with inactive SLE and active SLE, with corresponding serum BDNF values.

| | Inactive SLE(15) | Active SLE(35) |
|---|---|---|
| Age, mean (S.D.), years | 34.2 (13.7) | 30.6 (15.9) |
| Male/Female, n | 1/14 | 4/31 |
| Naïve patients, n | 2 | 24 |
| Disease duration, median, years | 3.7 | 3.4 |
| Mean prednisone dose, mg/day | 6.3 | 3.3 |
| Chloroquine, n (%) | 10 (67) | 19 (54) |
| Immunosuppressants[a], n (%) | 11 (73) | 20 (57) |
| Serum BDNF, median (range), pg/ml | 20,806 (11,774–29,100) | 12,385 (240–26,221) |

[a] AZA, LEF, MMF and cyclophosphamide. BDNF: brain-derived neurotrophic factor.

($r = -0.379$, $p = 0.011$; $r = -0.345$, $p = 0.022$; $r = -0.368$, $p = 0.014$, respectively). Table 1 shows the demographic and clinical characteristics of SLE patients as categorized into active and inactive groups.

### 3.2. Serum BDNF level

Serum BDNF level in SLE patients was significantly decreased when compared with the controls (14742.5 ± 7620.9 pg/ml vs. 33116.5 ± 7146.5 pg/ml, $p < 0.001$, Student's *t* test. Fig. 1A). Regarding activity status of SLE, the serum BDNF level in the inactive group was significantly increased when compared with the active group (20805.6 ± 5322.3 pg/ml, n = 15 vs. 12384.6 ± 7096.9 pg/ml, n = 35, $p < 0.001$, ANOVA and Tukey's test Fig. 1B). Thus, the serum BDNF level appeared to increase in parallel with improvement in SLE symptoms.

### 3.3. Comparison of serum BDNF levels in subgroups of SLE patients with and without systemic involvement

Serum BDNF level was significantly increased in non-NPSLE group when compared with NPSLE group (15818.6 ± 7332.6 pg/ml, n = 44 vs. 8200.3 ± 6111.5 pg/ml, n = 6, $p = 0.012$. Fig. 2A). As well, an increase in serum BDNF level was noticed in the SLE patients without LN compared to those with LN (17200 ± 6113.6 pg/ml, n = 25 vs. 12944.7 ± 7695.4 pg/ml, n = 25, $p = 0.042$. Fig. 2B). There was no meaningful difference found in serum BDNF levels between SLE patients without ILD and SLE patients with ILD (15182.5 ± 7325.4 pg/ml, n = 42 vs. 13686.9 ± 8723.1 pg/ml, n = 8).

### 3.4. Correlation between serum BDNF level and laboratory parameters

Table 2 listed the results of Pearson regression between serum BDNF level and laboratory parameters. Serum BDNF level in SLE patients was negatively correlated with SLEDAI score ($r = -0.619$, $p < 0.001$) and positively correlated with both C3 and CD3 + T cells ($r = 0.373$, $p = 0.010$; $r = 0.432$, $p = 0.003$. Fig. 3).

### 3.5. TrkB expression on the surface of CD3 + CD4 + and CD3 + CD8 + T lymphocytes

The percentages of TrkB expression on the surface of CD3 + CD4 + and CD3 + CD8 + T lymphocytes were described as CD3 + CD4 + TrkB +/CD3 + CD4 + and CD3 + CD8 + TrkB+/CD3 + CD8+, respectively. Fig. 4 presents an example of fluorescence activated cell sorter (FACS) dotplots for CD3+–CD4+, CD3+–CD8+, CD3 + CD4+–TrkB+, CD3 + CD8+–TrkB + . Statistical analysis revealed a significant increase in the percentages of TrkB expression on CD3 + CD4 + T cell surface (2.89 ± 2.85% vs. 0.65 ± 0.36%, $p < 0.001$, Student's *t*-test. Fig. 5A) and CD3 + CD8 + T cell surface (11.17 ± 7.39% vs. 3.27 ± 2.92%, $p < 0.001$, Student's *t*-test. Fig. 5B) in SLE patients when compared with the controls. The percentage of TrkB expression on the surface of total lymphocytes in SLE patients was also increased (2.53 ± 2.4% vs. 0.63 ± 0.34%,$p < 0.001$, Student's *t*-test. Fig. 5C). The percentage of TrkB on CD3 + CD8 + T cell surface was negatively correlated with corresponding serum BDNF level ($r = -0.477$, $p = 0.029$. Fig. 5D).

## 4. Discussion

In the present study we report, for the first time, the detection of TrkB expression on the surface of peripheral blood T lymphocytes in SLE patients. Individuals with SLE exhibited significantly lower serum BDNF level and higher TrkB expression on CD3 + CD4 + and CD3 + CD8 + T cell surface when compared with the controls. Increased TrkB expression can be considered as a likely compensatory mechanism for decreased serum BDNF levels.

Few previous studies have reported on changes serum BDNF levels in SLE. Even so, the results obtained were inconsistent. Tamashiro [30] and Zheng [38] reported that serum BDNF levels were negatively correlated with SLEDAI score, and positively correlated with C3 and lymphocyte counts; findings which are in agreement with ours. Nevertheless, they found that BDNF level in inactive SLE was higher compared with controls, and there was no difference between active SLE and controls. We observed the phenomenon that serum BDNF level unquestionably decreased after repeated thawing, leading to our speculation that BDNF protein is unstable in serum. Unlike in our study where all blood samples were collected freshly from study participants and analyzed within three months, samples for the control group in Tamashiro's study were obtained from the blood bank of the Clinical Hospital. Accordingly, it is highly likely that, with the possible long-term preservation of the blood samples, degradation of BDNF had occurred resulting in the observed lower level of serum BDNF in the controls. Our thoughts are supported by Zuccato [39] who also demonstrated that blood collection under different conditions and storage time might alter the BDNF level in blood. A case report by Ikenouchi-Sugita [32] showed that serum BDNF level in a NPSLE patient with irreversible organic brain change was consistently lower compared with that in the control. Furthermore, they also found that a transient raise in the BDNF level might indicate the possible reversal of brain damage. This observation is consistent with the results from our current study where it was apparent that serum BDNF level was lower in SLE

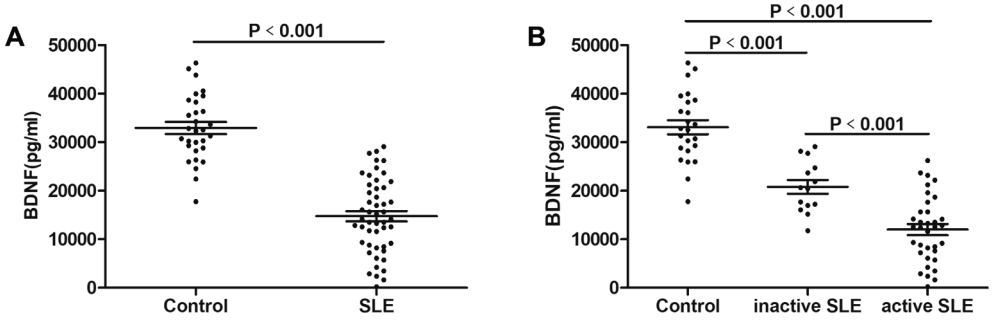


**Fig. 1.** Comparison of serum BDNF levels between controls and SLE patients (including inactive SLE and active SLE). (A) Serum BDNF levels in controls and SLE patients ($P < 0.001$). (B) Serum BDNF levels among the controls (n = 30), inactive SLE group (n = 15) and active SLE group (n = 35) ($P < 0.001$). BDNF: brain-derived neurotrophic factor.

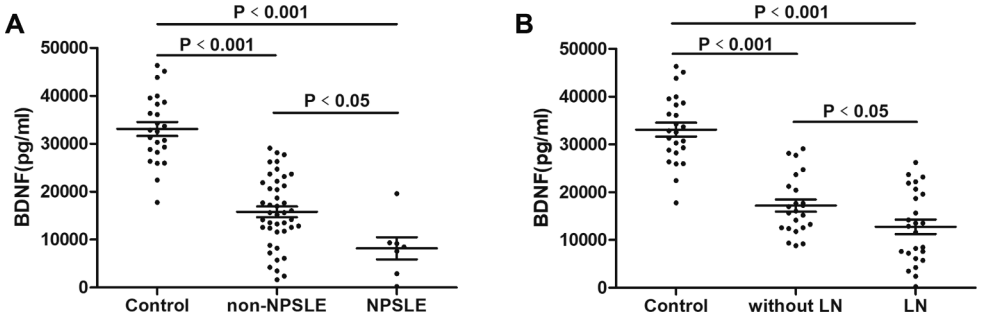


**Fig. 2.** Comparison of serum BDNF levels between SLE without systemic involvement and SLE with systemic involvement. (A) Comparison of serum BDNF levels among controls (n = 30), non-NPSLE group (n = 44) and NPSLE group (n = 6). (B) Comparison of serum BDNF levels among controls, SLE without LN group (25) and SLE with LN group (25). ANOVA and Turkey post hoc test. BDNF: brain-derived neurotrophic factor.

**Table 2**
Correlation between serum BDNF level and laboratory parameters.

| | BDNF | |
|---|---|---|
| Parameter | r | p |
| WBC ($10^9$/L) | 0.111 | 0.441 |
| LY ($10^9$/L) | 0.437** | 0.002 |
| PLT ($10^9$/L) | 0.628** | 0.000 |
| D-D (ug/ml) | −0.515** | 0.000 |
| Fib (g/L) | 0.240 | 0.101 |
| IgG (g/L) | −0.272 | 0.071 |
| IgA (g/L) | −0.074 | 0.616 |
| IgM (g/L) | −0.215 | 0.156 |
| C3 (g/L) | 0.373** | 0.010 |
| C4 (g/L) | 0.373** | 0.010 |
| CD4 + T | 0.428** | 0.003 |
| CD8 + T | 0.353* | 0.016 |
| CD3 + T | 0.432** | 0.003 |
| SLEDAI | −0.619** | 0.000 |

WBC: white blood cell; LY: lymphocyte; PLT: platelet; D-D: D-dimer; Fib: fibrinogen; IgG, A, M: immunoglobulin G, A, M; C3: complement3, C4: complement4; CD4 + T: CD4 + T lymphocyte; CD8 + T: CD8 + T lymphocyte; CD3 + T: CD3 + T lymphocyte; SLEDAI: systemic lupus erythematosus disease activity index. *: $p < 0.05$, **: $p < 0.01$, compared to controls, Pearson regression.

individuals. From the above-mentioned studies and our present work, serum BDNF level shows potential as a biomarker to monitor disease progress in SLE patients. To increase the robustness and reliability of the results from these studies, there is need to select drug-naïve SLE patients to avoid possible interferences due to drug treatment. Moreover, good laboratory practices including collection, handling and storage of blood samples are critical towards obtaining reliable results. A deficiency in our study is that there were fewer NPSLE patients, which possibly brought about bias in the analysis of inter-group differences.

We found that serum BDNF level was negatively correlated with SLEDAI score, and that higher BDNF values were present in inactive SLE patients compared to the active SLE patients. Increased serum BDNF level might suggest disease remission. However, conclusive results would require longer follow-up periods to ascertain this hypothesis. Vascular endothelial cells also synthesize BDNF and express TrkB [40,41] implicated in inducing neoangiogenesis in ischemic tissue [42]. D-D reflects impaired function of endothelial cells and formation of micro-thrombus in SLE. Our results showed a negative correlation between BDNF and D-D level, suggesting that serum BDNF level might be helpful in estimating the degree of vasculitis. A report by Fujimura [43] showed that BDNF level was lower (1.7 ± 1.7 ng/ml) in platelet-poor plasma, and platelets were thought to serve as a reservoir for circulating BDNF. As is well known, thrombocytopenia is a common feature in active SLE patients. It is, therefore, unsurprising that our results showed serum BDNF level to be positively correlated with PLT counts.

Serum BDNF level in SLE patients complicated with systemic involvements was much lower in this study. Previous articles have reported about local overexpression of BDNF and TrkB in spondyloarthritis [27] and usual interstitial pneumonitis (UIP) / idiopathic pulmonary fibrosis (IPF) [42,44–46]. Thus, this observation may be rationalized by the fact that the BDNF derived from lymphocytes is produced and consumed locally in the kidney, lung, nervous system and skin lesions. This proposition would need to be further proved by immunohistochemistry. For NPSLE, brain damage directly leads to the reduction in BDNF synthesis in the nervous system. There are reports on the transport of both BDNF [47] and glial cell line-derived neurotrophic factor [48] across the blood brain barrier. Additionally, it has been demonstrated, albeit in a rat model, that BDNF levels in brain and serum undergo similar changes during the maturation and aging process [49]. Furthermore, T cells in peripheral blood might penetrate damaged BBB, and the BDNF that was produced used to protect and repair the neurons [12,50,51]. Even though we only had six patients

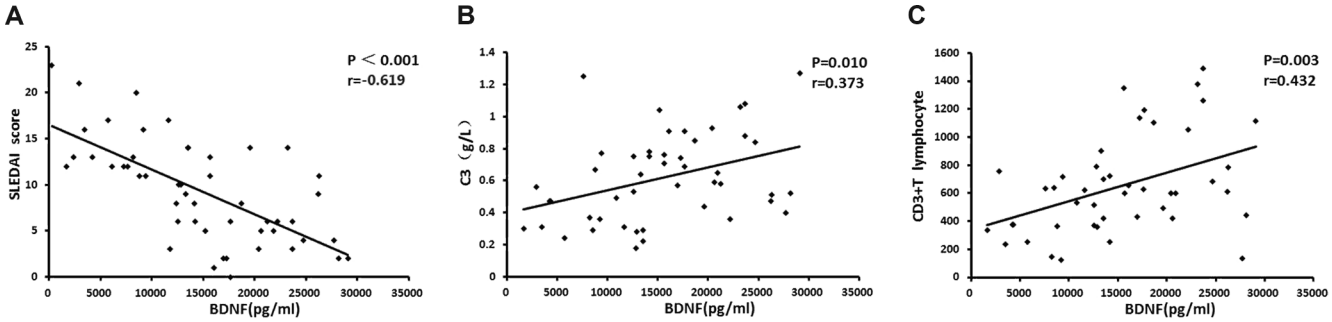


**Fig. 3.** There exits certain correlation between serum BDNF level and clinical parameters. (A) Negative correlation between serum BDNF level and SLEDAI score ($r = -0.619$, $p < 0.001$). (B) Positive correlation between BDNF level and C3 ($r = 0.373$, $p = 0.010$). (C) Positive correlation between BDNF level and CD3 + T lymphocytes ($r = 0.432$, $p = 0.003$). BDNF: brain-derived neurotrophic factor. SLEDAI: systemic lupus erythematosus disease activity index.

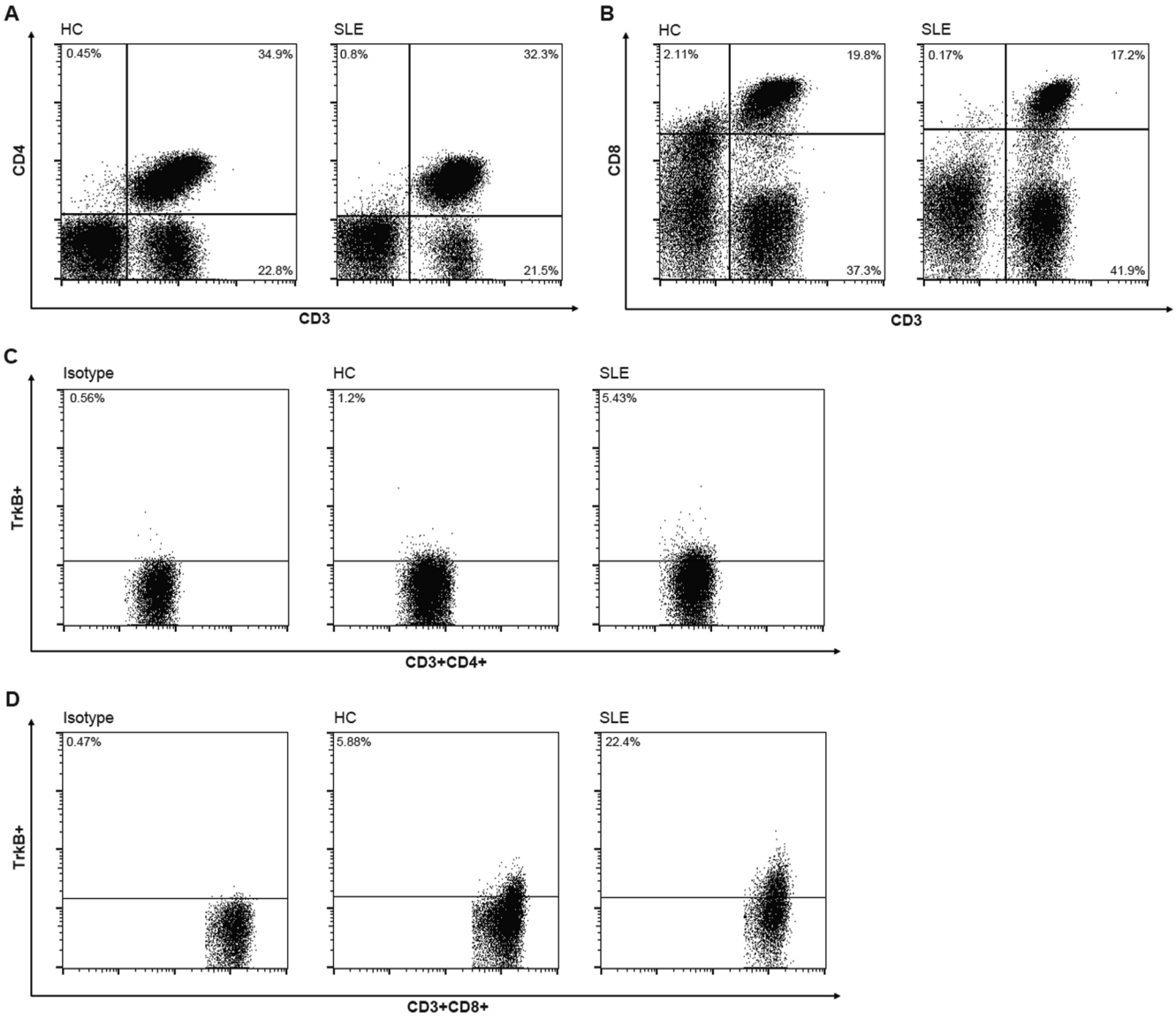


**Fig. 4.** Graphic representation of TrkB expression on CD3 + CD4+/CD3 + CD8 + T cells surface in controls and SLE patients. (A) FACS dot-plots of CD3+–CD4 + in control and SLE. (B) FACS dot–plots of CD3+–CD8 + in control and SLE. (C) FACS dot–plots of CD3 + CD4+–TrkB + for isotype, control and SLE. (D) FACS dot–plots of CD3 + CD8+–TrkB + for isotype, control and SLE.

with lupus encephalopathy, the observed low level of BDNF may serve as a biomarker indicating the presence of encephalopathy. In some studies, serum BDNF level was reported to be unaffected by use of corticosteroids [52,53] and immunosuppressants [54]. Taking these into accounts, lower serum BDNF level might suggest systemic involvement, especially for NPSLE. It is, therefore, possible that consistently lower serum BDNF level might be a useful biomarker to differentiate NPSLE from hormone-induced mental disorders.

Linker [55] reported that mice whose immune cells were deficient in BDNF displayed an attenuated immune response in the acute phase of experimental autoimmune encephalomyelitis (EAE). In addition, in a lentiviral approach, injection of BDNF-overexpressing T cells led to a less severe course of experimental autoimmune encephalomyelitis and direct axonal protection. Similarly, we speculate that decreased serum BDNF level in the active period of SLE may attenuate immune response and may be a protective mechanism. It is plausible that the role of BDNF in the pathophysiology of SLE might be complicated and remains to be elucidated. It is worth studying further about the initiation of intracellular signal transduction pathways after the binding of BDNF to TrkB in SLE and investigating whether BDNF has any effect on the differentiation of T cell subsets and secreting of inflammatory factors.

In conclusion, the present study showed that serum BDNF level was decreased in SLE individuals, especially in SLE with systemic involvement. On the other hand, TrkB expression on the T cell surface in SLE patients was significantly increased. It is possible that serum BDNF level may be a useful biological marker to reflect disease activity, systemic involvement and distinguish NPSLE from steroid psychosis.

## Declaration of Competing Interest

The authors declare no competing interest.

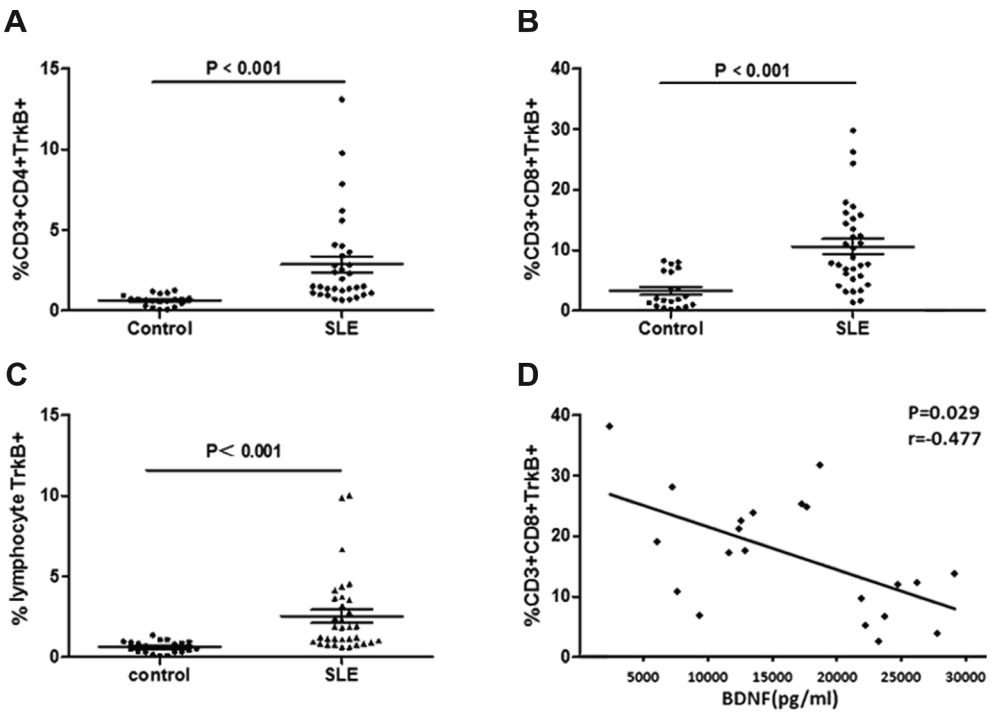


**Fig. 5.** TrkB expression on the surface of total lymphocytes and CD4+/CD8 + T lymphocytes was all increased. (A) The percentage of CD3 + CD4 + TrkB + cells/CD3 + CD4 + T cells in controls and SLE patients ($p < 0.001$). (B) The percentage of CD3 + CD8 + TrkB + cells/CD3 + CD8 + T cells in controls and SLE patients ($p < 0.001$). (C) The percentage of TrkB + cells/total lymphocytes in controls and SLE patients ($p < 0.001$). (D) Negative correlation between the percentage of CD3 + CD8 + TrkB + cells and corresponding serum BDNF level. BDNF: brain-derived neurotrophic factor.

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