ORIGINAL ARTICLE

Correlation between serum thyroxine and complements in patients with multiple sclerosis and neuromyelitis optica

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Abstract

OBJECTIVE: The aim of this study is to assess serum levels of thyroid hormones (TH) and complement C3, C4, CH50 in patients with multiple sclerosis (MS) and neuromyelitis optica (NMO), and investigate whether there is a correlation between serum TH and complement factors in these patients.

METHODS: One hundred and eighty-eight MS patients and sixty-five NMO patients were included in this study. The levels of serum TH were measured by magnetic antibody enzyme linking immunassay and complement C3, C4 and CH50 were determined by immunoturbidimetry.

RESULTS: The levels of TT4, complement C4 and CH50 in the serum were significantly higher in NMO patients than that in the MS. There were no significant differences in serum TT3, FT3, FT4 concentrations between NMO and MS patient. In MS patients, significant correlations between mean EDSS levels and serum C3 (r=0.223, p=0.014), and serum C4 (r=0.216, p=0.017) were found, and serum TT3 was positively correlated with serum CH50 (r=0.342, p=0.01). In NMO patients, the positive association between serum FT4 and C4 (r=0.533, p=0.006) were also found.

CONCLUSIONS: These results indicate that NMO patients has different serum TH and complement C3, C4 and CH50 levels from MS patients, and that serum TH levels is correlated with CH50 and C4 in these patients, suggesting TH may play a different role in modulating the complement activation in MS and NMO.

INTRODUCTION

Multiple sclerosis (MS) is considered to be a CD4+T-cell mediated disorder based on immune alterations in the blood and CSF as well as the pathologic features in the brain (Sospedra and Martin 2005). Recently, a causal role of complement in central nervous system (CNS) health and disease, particularly in the context of demeylinating conditions has become increasingly clear such as MS (Stahel and Barnum 2006; Lucchinetti *et al.* 2000; Gasque *et al.* 2002). Immunoglobulin-mediated tissue injury in MS is an attractive possibility given the capacity of antibodies to be highly selective in their target recognition. Mechanism may include antibody-dependent complement mediated cytotoxicity.

Neuromyelitis optica (NMO) is an inflammatory /demyelinating central nervous system (CNS) syndrome with a predilection for optic nerve and spinal cord. There is evidence showing that NMO is driven by humoral immunity mechanisms, including associations with systemic autoimmune diseases, therapeutic response to plasmapheresis, and immunological and immunopathological findings (Keegan *et al.* 2002; Lucchinetti *et al.* 2002; Wingerchuk *et al.* 1999). Immunoglobulin and complements are deposited in a characteristic vasculocentric rim and rosette pattern in active neuromyelitis optica lesions (Lucchinetti *et al.* 2002). Eosinophils and neutrophils commonly exist in the inflammatory infiltrates of active lesions of neuromyelitis optica (Mirsattari *et al.* 2001). Moreover, the detection of NMO-IgG, an autoantibody, in the serum of patients with neuromyelitis optica, distinguishes neuromyelitis optica from other demyelinating disorders (Lennon *et al.* 2004). NMO-IgG bind to aquaporin-4, which is the main channel that regulates water homoeostasis in the central nervous system (Amiry-Moghaddam and Ottersen 2003; Lennon *et al.* 2005). These features are not seen in classical MS.

The immune system may be divided into innate and adaptive immune systems, where the humoral immunity (complement) system is an important part of the innate immune system (Walport *et al.* 2001). The complement system is activated via classical pathway, alternative pathway and the plasma protein mannan-binding lectin (MBL). MBL can recognize patterns of carbohydrates presented on many microorganisms. Therefore, MBL participates in the pathology of infectious, autoimmune and cardiovascular diseases (Turner 2003; Hansen *et al.* 2004; Hansen *et al.* 1998; Collard *et al.* 2000).

At the present, a number of studies have indicated the existence of causal links between the endocrine and the immune system. Some studies reveal that MBL synthesis in human may be increased by TH (Sørensen *et al.* 2006; Riis *et al.* 2005). In this way, thyroid hormone may modulate complement activation, and plays a role in the pathogenesis of some autoimmune diseases.

In MS and NMO patients, peripheral circulating TH and complements enter into CNS across the destructed blood-brain barrier and become the important source of complements in CNS. The aim of this study is to assess serum levels of thyroid hormones (TH) and complement C3, C4, CH50 in multiple sclerosis (MS), neuromyelitis optica (NMO) patients, and investigate whether there is a correlation between TH and complement factors in these patients.

METHODS

Patients

Study material consisted of 127 patients with MS and 40 patients with NMO. All the patients were admitted to the Department of Neurology, the third affiliated hospital of Sun Yat-sen University, between August 2000 and July 2007. MS patients comprised 76 women and 51 men. The mean age was 33.7±13.4 years and mean EDSS (Kurtzke's Expanded Disability Status Scale score) (Kurtzke 1983) levels was 3.6±2.1. All the patients in this study matched McDonald diagnostic criteria for MS (Polman *et al.* 2005). NMO patients met the criteria proposed by Wingerchuck (Wingerchuck *et al.* 1999). The group of patients with NMO comprised of 37 women and 3 men. The mean age was 33.5±15.4 years and mean EDSS levels was 4.9±2.0. None of the patients had thyroid autoimmunity or other diseases with hormone disturbance, and no patients were treated with corticosteroids or other immunosuppressives within two months. All patients experienced their attacks when were sampled serum and autoimmune thyroid disease was excluded in all patients by measurement of serum thyrotrophin receptor antibody (TRAb). All clinical detections had been approved by the local ethics committee and all patients had learned about our examination and agree with it.

Thyroid hormonal and complements analysis in serum

All the patients with MS and NMO were subjected to physical examinations and their medical history was taken. Blood for thyroid hormonal analysis was collected in the morning (07:00–08:00h), and the blood was subjected to centrifugation for 20 minutes (2000 g/min.).

For assessment of thyroid function, total T4 (TT4), total T3 (TT3), free T4 (FT4) and free T3 (FT3) were evaluated in serum samples by highly sensitive MAIA (magnetic antibody enzyme linking immunoassay) following the procedure of the manufacturer's instructions. The sensitive limits of the assays were 7.5 nmol/l, 6.435 nmol/l, 0.129 pmol/l and 0.462 pmol/l for TT3, TT4, FT4 and FT3 respectively.

Complement C3, C4 and CH50 concentrations were measured by immunoturbidimetry following the procedure of the manufacturer's instructions. Goat anti-human C3C and C4C complement antibodies were from DiaSys (Germany), and goat anti-human DNP antibody was from Wako (Japan). The sensitive range of the assays is 0.01–5.00g/L and 0.006–0.90 g/L for C3C and C4 respectively. The minimum detectable level of CH50 is estimated to be 10 U/mL. All the patients had the detection of serum C3, C4, CH50, and 61 MS patients and 25 NMO patients had the examination of serum thyroxine among the total.

Statistical analysis

Data were compared using Student's *t-test* and Mann–Whitney's tests using the SPSS 13.0 statistical package. Statistical significance was assumed when the probability (p) was less than or equal to 0.05. The relationships between variables were derived using Spearman's (rho) correlation coefficients. Correlation analysis was performed with Spearman's rho analysis. Serum thyroid hormones and complements in MS and NMO patients

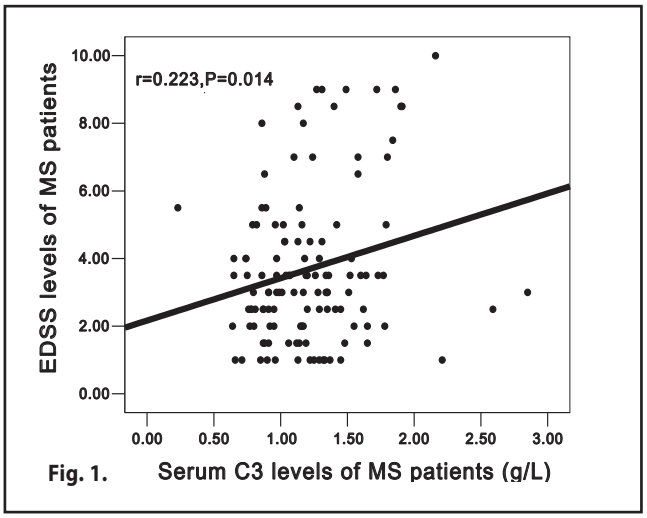


Fig. 1.

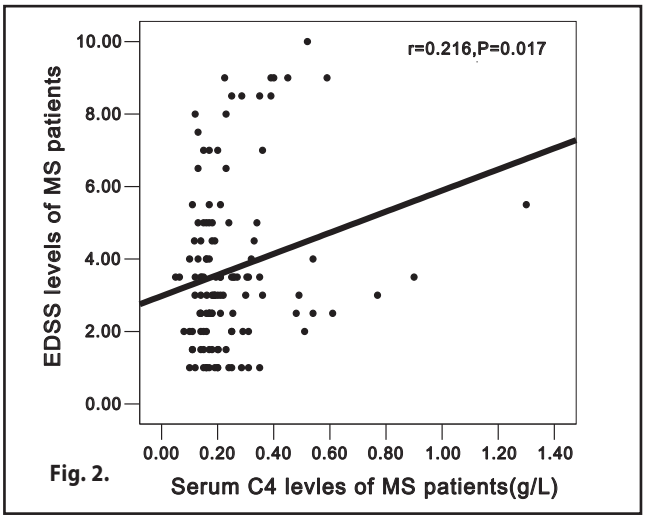
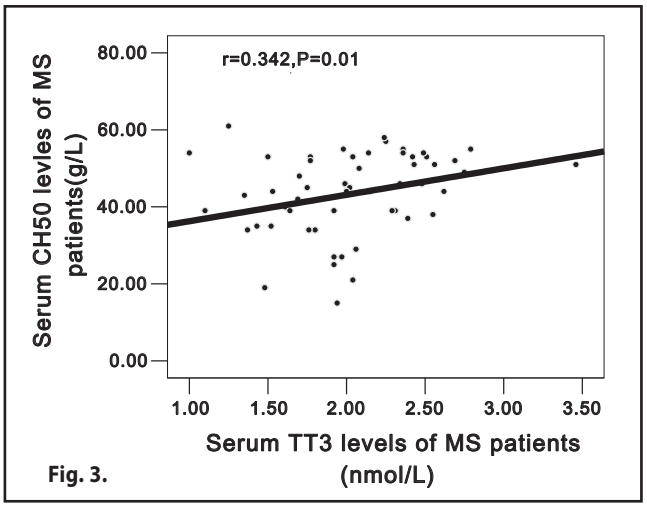


Fig. 2.



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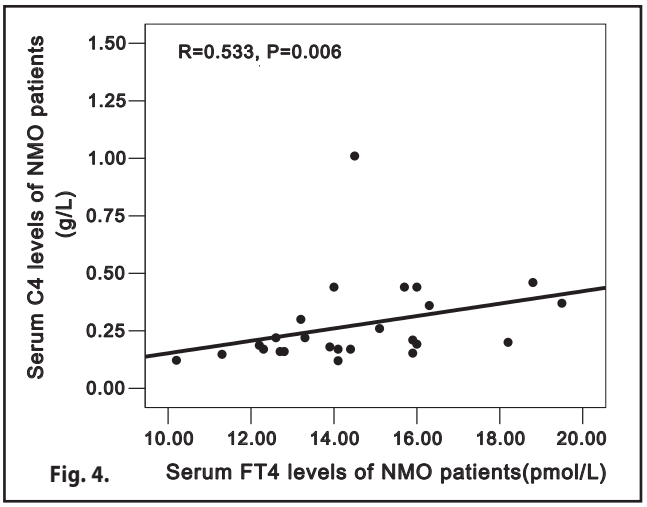

Fig. 4.

did not comply with normal distribution, and when comparing these parameters Mann–Whitney's U-test for unpaired comparisons was used. The results corresponding to serum complements and thyroid hormones in patients are expressed as mean±SE.

RESULTS

The characteristics of patients are presented in Table.1. The levels of complement C4 and CH50 in the serum were significantly higher in NMO patients than that in MS patients. The levels of serum TT4 were also higher in NMO patients compared to MS patients. In contrast, there were no significant differences in serum levels of TT3, FT3 and FT4 between NMO and MS patients. (respectively p=0.985, 0.802, 0.625)

NMO patients had much higher EDSS levels (4.9±2.0) than in MS patients (3.6±2.1). Significant positive correlations were found in MS patients between the mean EDSS levels and serum C3 (r=0.223, p=0.014) **(Fig 1)**, and serum C4 (r=0.216, p=0.017) **(Fig 2)**. However, such correlations were not detected in NMO patients. In addition, we found that serum levels of TT3 were positively correlated with the levels of CH50 in MS patients (r=0.342, p=0.01) **(Fig 3)**, and that serum FT4 was positively correlated with serum levels of complement C4 (r=0.533, p=0.006) in NMO patients **(Fig 4)**.

Table 1. Serum humoral immunity components and thyroxin levels in MS and NMO patients

	MS	NMO
Age	33.7±13.4	33.5±15.4
Male/female	51/76	3/37
EDSS	3.6±2.1	4.9±2.0**
TT3(nmol/l)	2.0±0.52	2.0±0.27
TT4(nmol/l)	104.98±19.46	119.60±19.09*
FT3(pmol/l)	4.00±0.73	4.01±0.72
FT4(pmol/l)	13.86±2.25	14.52±2.27
C3**(g/L)**	1.21±0.39	1.36±0.56
C4**(g/L)**	0.25±0.17	0.64±2.40***
CH50**(g/L)**	40.40±10.90	46.04±11.43****

*p=0.002; p<0.01; ** p=0.000; p<0.001; *** p=0.017; p<0.05; **** p=0.003; p<0.01,

DISCUSSION

The complement cascades are activated via three pathways: the classical, alternative, or lectin pathways. The lectin pathway is initiated when mannose-binding lectin (MBL) binds to monosaccharides on the surfaces of bacteria and parasites. MBL then interacts with MBL associated serine proteases (MASP)-1, 2 and 3, which in turn becomes active and forms C3 convertase by cleaving C2 and C4 (Wallis 2002). These pathways converge at the level of C3 cleavage and proceed to the assembly of the terminal, membrane attack pathway. When MBL recognizes patterns of carbohydrates, such as that presented on many microorganisms, activation of the complement system occurs. This is a beneficial activity, but many different products during such activation are potentially harmful for the body itself (Thiel *et al.* 1997). Experimental data have demonstrated that MBL is involved in complement-mediated injuries induced by altered self-tissues after ischaemia and reperfusion of kidney, heart or intestinal tissue (Hart *et al,* 2005). The changes in MBL concentration are mainly caused by TH levels, rather than autoimmunity (Riis *et al.* 2005). The mechanisms whereby TH increases blood concentrations of MBL are uncertain. Presumably, increased levels of TH activate hepatic MBL synthesis via transcriptional regulation of target genes (Yen 2001). In this way, TH may modulate complement activation, and play a role in the pathogenesis of thyroid diseases. Previous studies have shown that the MS patients had higher T4 and lower T3 levels in serum than controls, suggesting the existence of thyroid dysfunction in MS. However, few reports pay attention to the serum TH in NMO patients. So we investigate the serum TH levels and the correlation with complements in NMO patients.

At first, Glucocorticoid is mostly available treatment in our clinical practice, such as Prednisolone (Pfzer Manufacturing Belgium NV). According to the manufacturer's instructions, the half-life of Prednisolone is 12–36 hours. The activity of Prednisolone is still existing through its effect on hypothalamic-pituitary-adrenal axis suppression (HPA). As previous studies, the hypothalamic-pituitary-thyroid (HPT) axis plays the important role in regulation of TH levels (Martin *et al.* 2006, Goncharova and Lapin 2004, Arancibia *et al.* 1996). Up to now, few people report that the ectogenic glucocorticoid (GC) directly affects on HPT axis. So we consider ectogenic Prednisolone treatment has few effects on serum TH after mean two months since withdrawal. Secondly, the higher serum levels of T4 in NMO show us the more active TH metabolism. Furthermore, hypothalamic localization of aquaporin-4 and NMO-IgG bind to aquaporin-4 (Lennon *et al.* 2004) indicate that hypothalamus is the predilection site of NMO patients as previous study (Vernant *et al.* 1997). Nevertheless, MRI documented hypothalamic lesions in MS are uncommon and clinical-imaging correlation in this context is rare (Tsui *et al.* 2002). Therefore, it is interesting to speculate whether lesions in NMO that selectively involved the hypothalamic pathways commonly lead to hypothalamic axis dysregulation including HPT axis and more active TH metabolism in serum.

Recently, B cells (Archelos *et al.* 2000) and activated complement (Storch *et al.* 1998) are observed in active multiple sclerosis lesions. Furthermore, immunoglobulin and complements are deposited in a characteristic vasculocentric rim and rosette pattern in active neuromyelitis optica lesions (Lucchinetti *et al.* 2002). Active neuromyelitis optica lesions are distinctly different because their vasculocentric distribution of immune complexes is related with the normal expression of aquaporin-4 in the endfeet of astrocytes (Roemer *et al.* 2007). According to these researches, the association of complements with the development of MS and NMO has been confirmed.

In the study, we compared the serum complements concentrations between MS and NMO. Compared with MS patients, much higher serum C4 and CH50 concentrations in NMO patients reflect increasing activity of complement pathways in NMO patients than MS patients, which seem to support that humoral-mediated autoimmune response play the major role in the pathogenesis of NMO. And we found increasing serum TT3 was correlated with the increasing serum CH50 concentrations in MS patients, serum FT4 was correlated with the increasing serum C4 concentrations in NMO patients. The results showed TH play an important role in humoral immunity underlying the pathogenesis of MS and NMO patients. Firstly, the remarkable effects of TH on complements play the important role of complement activation in inflammatory demyelination. Morgan have demonstrated the activated C5b-9 complex by myelin and oligodendrocytes plays a pro-inflammatory role in the acute phase of EAE and in the MS when a breakdown has occurred in the blood–brain barrier (Morgan *et al.* 2004). Secondly, T3 and T4 significantly promoted MBL synthesis in a dose-dependent manner (Sørensen *et al.* 2006; Riis *et al.* 2005). So T3 and T4 modulated the activation of complements via enhancement of serum MBL and initiation of lectin pathway underlying the pathogenesis of MS and NMO. Finally, a comparable effect of T4 on serum MBL was seen at a concentration 100 times higher than that needed of T3, which is in concordance with T3 being the most potent of the two in vivo (Lin *et al.* 2003). According to these, much higher serum T4 concentrations in NMO patients may be in favour of the initiation and modulation of complements. So far, no study about the detection of serum MBL levels in MS and NMO patients is reported. Further study will be needed about the expression of MBL, serum concentrations of MBL, the relationship between serum MBL and TH, complements in MS and NMO patients.

It is well known that EDSS levels are used to assess the severity and activity of MS and NMO patients (Kurtzke 1983). Because of the important role of humoral immu-

nity in the pathogenesis of MS and NMO, we attempted to demonstrate the associations between complements and diseases activity. Our results conclusively showed that there were increased serum complements (C3, C4) in MS patients with much more disease severity. The significantly positive correlation showed the humoral pathological changes in MS directly affected on disease severity. So a number of therapeutic approaches aimed at removing disease-relevant humoral immune responses in MS have been done or are continuing to be evaluated in MS, such as therapeutic plasma exchange and intravenous (IV) Ig.

In conclusion, our results suggest humoral-mediated autoimmune response plays an important role in the pathogenesis of NMO and MS, and serum complements are correlated to disease activity and severity of MS patients. Serum TH can cause the humoral-change in MS and NMO patients. But much more work should be done to find how TH regulates humoral-mediated autoimmune response in MS and NMO patients.

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