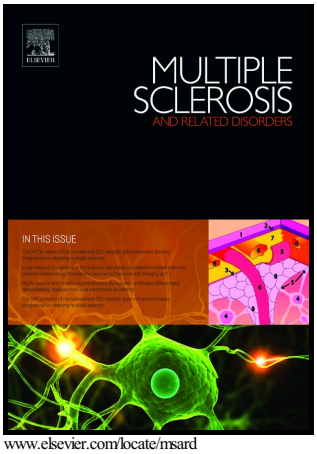

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Association of serum gamma-glutamyltransferase and C-reactive proteins with neuromyelitis optica and multiple sclerosis

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ABSTRACT

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Background

Many studies have demonstrated that serum gamma glutamyltransferase (GGT) within normal range might be an early marker of oxidative stress. However the role of GGT in neuromyelitis optica (NMO) and multiple sclerosis (MS) is unknown.

Methods

We assessed the correlations among GGT and C-reactive protein (CRP) levels, as well as clinical characteristics of NMO and MS. Serum GGT and CRP levels were measured in 106 NMO patients, 87 MS patients, 79 patients with non-inflammatory neurological diseases (Parkinson disease) and 80 healthy controls (HC). Clinical parameters, blood-brain barrier (BBB) index and Delpech index of MS and NMO were also investigated.

Results

We found that NMO patients had higher serum GGT and CRP levels within their normal ranges compared to MS, PD, healthy controls. NMO patients exhibited significantly higher EDSS scores than MS patients. The BBB index in NMO patients was significantly higher than that in MS patients. Significant correlations existed between serum GGT and CRP levels and EDSS scores, BBB index in NMO and MS patients.

Conclusion

Elevated GGT and CRP levels within their normal ranges in NMO and MS may be associated with inflammatory response, oxidative stress and BBB disturbance in the diseases. Further study into the underlying pathophysiology of this relationship is warranted.

Abbreviations

GGT, gamma-glutamyltransferase; CRP, C-reactive protein; MS, multiple sclerosis; NMO, neuromyelitis optica; PD, Parkinson disease; EDSS, expanded Disability Status Scale; NMO-IgG, neuromyelitis optic-immunoglobulin; AQP4, aquaporin 4; BBB, blood-brain barrier.

Keywords: gamma-glutamyltransferase; C-reactive protein, neuromyelitis optica; multiple sclerosis; oxidative stress; blood-brain barrier.

1. Introduction

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the central nervous system (CNS) that can cause severe optic neuritis and myelitis (Kira 2011). Multiple sclerosis (MS) is also an inflammatory demyelinating disease of the CNS (Kira 2003). In Asia, NMO is more common than MS. The distinction between NMO and MS has long been unclear. For many decades NMO was considered a variant of MS. However some studies have shown that inflammatory NMO profiles primarily present as eosinophils/neutrophils and autoantibody reactions (Correale and Fiol 2004; Weinshenker 2007), whereas MS has T-lymphocyte and mononuclear macrophage reactions as the primary immunopathogenesis (Barnett et al. 2006). In addition a majority of NMO patients develop auto-antibodies (NMO-IgG) against aquaporin 4 (AQP4) in the CNS (Lennon et al. 2004). As a result Weinshenker et al suggested that NMO was distinguishable from MS in clinical, imaging, serological, and immunopathological profiles (Weinshenker 2003).

A series of epidemiological studies (Lee et al. 2004; Lee and Jacobs 2005; Lee et al. 2003; Lee et al. 2004) have suggested serum gamma glutamyltransferase (GGT) within its normal range might be an early marker of oxidative stress. C-reactive protein is proven to be a classical marker of inflammation. Lee et al (Lee and Jacobs 2005) argued that oxidative stress leads to an inflammatory response and elevation in GGT might occur before elevation in CRP. CRP values were similar in patients with

MS and in healthy controls but higher during MS relapses than in remission (Soilu-Hanninen et al. 2005).

Although the relationship between cellular GGT and serum GGT is not known, cellular GGT has been known to play an important role in antioxidant defense systems (Kugelman et al. 1994). Paradoxically, cellular GGT may also be involved in the generation of reactive oxygen species in the presence of transition metals (Drozdz et al. 1998; Glass and Stark 1997) and glutathione metabolized by GGT initiates an oxidative process that leads to a radical-rich environment and oxidative damage (Stark et al. 1994). In addition GGT expression regulates reactive oxygen species (ROS) in T-lymphocytes and modulates Fas-induced damage by altering NF-kappa B activity (Carlisle et al. 2003). As we know, oxidative stress appears to be a key component of many reactions associated with chronic inflammation, and chronic oxidative stress is thought to result in damage to DNA, lipids, proteins, and other molecules which may contribute to the development and progression of chronic diseases including diabetes (Xu et al. 2011), cardiovascular disease (Holvoet et al. 2007), cancer (Strasak et al. 2008), MS (Gonsette 2008a, b), and NMO (Penton-Rol et al. 2009). Previous studies have shown that serum GGT level was increased in some chronic diseases such as diabetes (Lee et al. 2003), cardiovascular disease (Lee et al. 2007) and cancer (Strasak et al. 2008). However little is known about GGT and CRP levels in idiopathic inflammatory CNS diseases such as NMO and MS. The present study aimed to explore the combined effects of GGT and CRP in NMO and MS.

2. Methods

2.1 Study subjects

The present study was performed at the MS Clinical Center, Department of Neurology, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, P. R. China. From 2007 to 2011, serum samples were collected from 352 participants. In order to investigate serum GGT levels in patients with non-inflammatory neurological disorders we also enrolled Parkinson disease (PD) patients as a control group. Demographic and clinical characteristics of NMO and MS patients, as well as PD patients and healthy controls are presented in Table 1. MS was identified in accordance with McDonald's criteria (Polman et al. 2005), and NMO was defined according to Wingerchuk's criteria (Wingerchuk et al. 2006). All patients were scored using the Expanded Disability Status Scale (EDSS).

2.2 Ethics Statement

The present study's protocol was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (No.2007-33), and all participants involved in this study provided written informed consent. Consent was given both in writing and verbally for measuring the Expanded Disability Status Scale (EDSS)

scores and all measurements were performed by an experienced neurologist who was blinded to the diagnostic categorization. The measurement procedure was taken as defined by Kurtzke (Kurtzke 1983). Since those measurements benefit the therapies of NMO and MS patients involved in this study, the ethics committees also approved this consent procedure.

2.3 Anti-AQP4 antibody serum testing

Serum from 111 NMO patients was tested for the presence of anti-AQP4 antibodies using a commercial sampling kit (Euroimmun, Lübeck, Germany) in accordance with the manufacturer's instructions.

2.4 Blood-brain barrier (BBB) index and Delpech index

Samples of cerebral spinal fluid (CSF) and matched serum were obtained from MS patients and NMO patients. Three MS patients refused lumbar puncture. The concentrations of IgG and albumin (Alb) in paired serum and CSF were measured by nephelometry, and the BBB index and Delpech index were calculated according to the following formulas: BBB index formula=1000 Alb csf/Alb serum and Delpech index formula= (IgG csf /IgG serum)/ (Alb csf/Alb serum) .

2.5 Biochemical measurements

Serum GGT level and CRP level were measured using an autoanalyser (Clinical Analyzer 7180-ISE, Hitachi High Technologies, Tokyo, Japan). Venous blood samples for serum GGT measurements were obtained from all subjects. Levels of serum GGT > 50U/L were determined to be above laboratory pathology cutpoint in our hospital. Levels of serum GGT > 50U/L, and alanine transaminase (ALT) > 45 U/L were determined to abnormal liver function.

Exclusion criteria included treatment with acetylsalicylic acid, thiazide diuretics, ibuprofen, and other drugs that could influence liver enzyme levels, as well as subjects with diabetes or liver, heart, or renal disease and alcohol intake. All samples were taken before intravenous methylprednisolone (IVMP). Fulfillment of inclusion and exclusion criteria was confirmed retrospectively by a review of medical records by the neurologist specialized in demyelinating diseases.

2.6 Statistical analysis

All continuous variables were presented as the mean（± standard deviation) if the data was normally distributed or as medians (min, max) if the data was not normally distributed. The categorical variable (gender) was shown as a percentage. The effect of age on serum GGT levels for different groups was analyzed by covariance analysis.

The comparison between serum GGT levels of the NMO, MS, PD, and healthy control subjects was performed using covariance analysis with age as the covariant. Since serum GGT level has been shown to be dependent on gender, patients within each group were divided into two subgroups according to gender. In order to compare serum GGT levels in NMO patients with anti-AQP4 antibody seropositive and with anti-AQP4 antibody seronegative, we also used covariance analysis with age and gender as the covariant. A Spearman's rank correlation coefficient was used to evaluate the association of age, disease duration, annualized relapse rate, EDSS score and serum GGT level in NMO and MS patients. SPSS 16.0 (Chicago IL, USA) was used for the statistical analyses. Any p values of less than 0.05 were regarded as statistically significant.

3. Results

Mean EDSS scores in the NMO group were significantly higher than in the MS group (3.8 *vs* 2.9, $P < 0.001$, Table 1). No significant differences were detected in age, disease duration, or annualized relapse rates between the MS and NMO groups. There were significant differences in annualized relapse rates between AQP4-seropositive NMO patients and AQP4-seronegative NMO patients, and serum GGT levels and BBB index in seropositive NMO-IgG NMO were higher than that in seronegative

NMO-IgG NMO patients, however the differences were not statistical significant (Table 2).

Serum GGT levels and CRP were significantly higher in NMO patients than that in MS, PD and healthy controls (Figure. 1A, C; Table 1). And serum GGT and CRP were also higher in MS than that in PD, healthy controls, but the differences were not significant. And the differences of serum ALT within normal range between NMO, MS, PD and healthy controls were not significant (Figure 1B).

In order to compare the BBB integrity in NMO and MS groups, we investigated the BBB index and Delpech index. The BBB index in the NMO group was significantly higher than that in the MS group (6.50 ± 4.41 vs 5.33 ± 3.03, p=0.038, Figure 2A), and the Delpech index in the NMO group was also higher than that in the MS group, however the difference was not statistically significant (0.54 ± 0.38 vs 0.46 ± 0.21, p=0.108) (Figure 2B).

Furthermore, serum GGT and CRP levels were significantly higher in female NMO patients than in female MS patients, female PD patients or female healthy controls (Figure. 3A, B), serum GGT and CRP levels were significantly higher in male NMO patients than that in male PD. However there was no significant difference in GGT and CRP levels for the male cohort between NMO, MS or healthy controls (Figure. 3).

In NMO patients there was a significant correlation between serum GGT levels and CRP level (r_s =0.502, p<0.001, Figure 4A), EDSS scores (r_s = 0.532, p<0.001, Figure 4B), BBB index (r_s = 0.585, p<0.001, Figure 4C), age (r_s = 0.359, p < 0.001, Figure 4D), but no significant correlations between GGT levels and annualized relapse rate, disease duration or Delpech index. In MS patients there was significant correlation between serum GGT levels and CRP level (r_s = 0.221, p =0.04, Figure 4E), EDSS score (r_s = 0.310, p =0.004, Figure 4F), BBB index (r_s = 0.391, p <0.001, Figure 4G), disease duration (r_s = 0.234, p = 0.029, Figure 4H), but correlations between GGT level and age, annualized relapse rate, or Delpech index were not significant.

4. Discussion

In the present study EDSS scores were significantly higher in NMO patients compared to MS patients as a result of more severe blindness and paralysis. This was reflected by more severe disability in NMO which was consistent with previous studies (Li et al. 2010; Weinshenker 2007).

GGT is a well-established serum marker for liver disease. However, elevated GGT is associated to increased risk to a multitude of diseases and conditions, including cardiovascular disease, diabetes, metabolic syndrome (MetS) and so on (Goldberg 2010; Koenig and Seneff 2015). Elevated GGT levels could lead to prooxidant activity, particularly in the presence of iron or copper (Milnerowicz et al.

2014; Corti et al. 2009). Furthermore, elevated GGT levels could damage to red blood cell membranes, then caused the release of these potentially toxic transition metals, which could further result in chain, prooxidant reactions (Aberkane et al. 2002). And increased prooxidation levels can contribute to downstream cell, tissue, and DNA damage caused by oxidative and nitrosative stress and the generation of deleterious reactive oxygen species or nitric oxide (Stefano and Kream 2015). Therefore, GGT is a marker of oxidative stress, and an elevated serum GGT level within normal range is thought to be associated with and oxidative stress and inflammation (Koenig and Seneff 2015; Lee and Jacobs 2005, 2015; Lee et al. 2008). Similarly, increased plasma CRP is correlated with myocardial infarction, PD, MS, and ischemic stroke (Soilu-Hanninen et al. 2005; Zhang et al. 2011; Kjaergaard et al. 2010). In the present paper, serum GGT and CRP levels were higher in patients with NMO and MS than that in patients with PD and healthy controls. And we also found serum GGT were positively associated with CRP, BBB index in NMO and MS patients. The results demonstrate that the elevated serum GGT and CRP may be associated with oxidative stress and inflammatory response in NMO and MS. Therefore we inferred that elevated serum GGT within normal range could serve as a marker for more oxidative and inflammatory stress and severe BBB destruction in NMO and MS.

Furthermore, NMO patients have significantly higher GGT level and BBB index than that in MS. Many studies have shown that oxidative reactions and inflammatory profiles in NMO patients are different from MS patients (Correale and Fiol 2004;

Gonsette 2008a; Weinshenker 2007; Wingerchuk and Lucchinetti 2007). In addition the mechanism of blood-brain barrier (BBB) destruction in NMO patients may differ from that in MS patients (Lennon et al. 2004; McQuaid et al. 2009; Minagar and Alexander 2003; Pittock et al. 2006; Vincent et al. 2008). The BBB index, which may reflect BBB destruction, was significantly higher in the NMO patients than that in the MS patients in our present study. On the other hand, many previous studies have shown that GGT, which was known as a marker for BBB function, played an important role in maintaining BBB integrity (Beuckmann et al. 1995; Kuchler-Bopp et al. 1999; Meyer et al. 1991; Ramsauer et al. 1998). Our results suggested that an elevated serum GGT level within normal range in NMO patients may reflect more severe oxidative and inflammatory reactions, as well as BBB dysfunction in NMO patients.

Aquaporin 4 (AQP4) is a water channel expressed by astrocytes and is localized at astrocyte footpads adjacent to endothelial cells, and exerts effects on the BBB. Some studies have suggested that anti-AQP4 antibodies (NMO-IgGs) might participate in pathogenic mechanisms of BBB dysfunction in NMO (Lennon et al. 2004; Pittock et al. 2006; Vincent et al. 2008). NMO-IgGs exhibit functional effects on cellular components of the neurovascular unit and also promote BBB opening and granulocyte recruitment in NMO (Vincent et al. 2008). In our study, we found annualized relapse rate in seropositive NMO-IgG NMO patients was significantly higher than that in seronegative NMO-IgG NMO patients, suggested that seropositive NMO-IgG NMO

patients may more likely to relapse. And serum GGT levels and BBB index in seropositive NMO-IgG NMO were higher than that in seronegative NMO-IgG NMO patients, though differences were not statistical significant.

In the present study we also found serum GGT and CRP levels were significantly higher in female NMO patients than in female MS, PD patients or female healthy controls. And female MS had higher GGT levels than that in female healthy controls.

Correlation was measured to determine the association between clinical variables and serum GGT and CRP in NMO patients. First, serum GGT was significantly positively associated with CRP in NMO, MS. This was compatible with the hypothesis that GGT was associated with the inflammatory response. Second, serum GGT levels and CRP level both had significant correlation with EDSS and BBB index in NMO and MS patients. We speculated that serum GGT and CRP might be associated with the BBB destruction, progression and outcome in NMO and MS patients.

To the best of our knowledge, this is the first study to analyze serum GGT levels in NMO and MS patients. However several limitations existed in the study. Firstly, the sample size was relatively small [NMO (n = 106), MS (n = 87), PD (n= 79) and healthy controls (n = 80)]. Secondly, biases are difficult to prevent with retrospective studies. Thirdly, the major limitation of this report is the fact that it is essentially an initial observational exploratory study without corroborating experimental evidence.

In the future we will move on to study GGT in CNS inflammation in the animal model.

5. Conclusions

Results from the present study suggested that serum GGT and CRP levels were elevated within their normal ranges in NMO and MS patients versus controls. We speculated that elevated serum GGT and CRP level within their normal ranges may be associated with the inflammatory response, oxidative stress and BBB destruction in NMO and MS patients. Further studies regarding the role of GGT in NMO and MS may be warranted.

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Conflict of interest statement

None.

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Figure 1 Serum GGT, ALT, CRP level in NMO and MS patients, PD patients, as well as healthy controls. A. Serum GGT level in NMO patients was significantly higher than in MS patients, PD patients, healthy controls respectively. Serum GGT level in MS patients was not significantly higher than in PD patients and healthy controls. Values represent mean ± SD. **B.** The differences of serum ALT levels between NMO, MS, PD and health controls were not significant. Values represent mean ± SD. **C.** Serum CRP in NMO levels were significantly higher than that in MS, PD and controls. Values represent median with range. $^{*}p < 0.5$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure 2 BBB index and Delpech index in NMO and MS. The BBB index in the NMO group was significantly higher than that in the MS group (Figure 2A), and the Delpech index in the NMO group was also higher than that in the MS group, however, the difference was not statistically significant (Figure 2B). Values represent mean ± SD.

Figure 3 Serum GGT level and CRP in male and female NMO, MS, PD, and healthy controls. A. In the female cohort, serum GGT levels in NMO patients were significantly greater than that in MS, PD and healthy controls respectively. And serum GGT levels in female MS patients were significantly higher than that in female healthy controls. Values represent mean ± SD. B. In female cohort, serum CRP levels were significantly higher in NMO than that in MS, PD and health controls. Values represent median. $^{*}p < 0.5$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Figure 4. Association between serum GGT and CRP, EDSS and BBB index in NMO and MS patients. ABCD, serum GGT levels versus CRP level. EDSS scores, BBB index, age in NMO patients. **EFGH**, serum GGT levels versus CRP level, EDSS score, BBB index, disease duration in MS patients.

Table 1 Demographic and clinical parameters of NMO, MS, PD and Healthy controls

Clinical parameters	NMO (n = 106)	MS (n = 87)	PD (n = 79)	HC (n = 80)	p
Male (n, %)	20 (18.9)	32 (36.8)	41 (51.9)	33 (41.2)	-
Age (years, mean, range)	36.7 (13 - 65)	34.2 (9 - 68)	62.2 (39 - 98)	36.9 (12 - 80)	0.185
Disease Duration (months, median, range)	18 (0.1 - 240)	12 (0.2 - 420)	60 (4 - 180)	-	0.697
Annualized relapse rate (median, range)	2 (1 - 12)	2 (1 - 13)	-	-	0.805
EDSS scores (mean, range)	3.8 (1 - 8.5)	2.9 (0 - 9.5)	-	-	0.005

Serum GGT level (U/L, mean, range)	29.26 (8 - 50)	24.66 (9 - 48)	21.05 (8 - 49)	21.05 (10 - 50)	0.011
Serum ALT level (U/L, mean, range)	20.86 (8 - 45)	20.43 (7 - 41)	20.87 (7 - 40)	20.11 (10 - 44)	0.978
Serum CRP level (mg/L, median, range)	1.85 (0 - 21.2)	1 (0 - 19.6)	0.9 (0 - 5)	0.75 (0 - 13.3)	<0.001

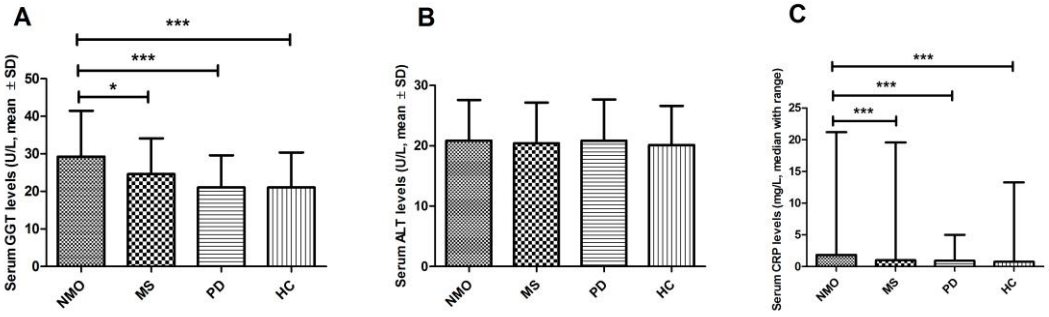
MS, multiple sclerosis; NMO, neuromyelitis optica; PD, Parkinson disease; HC, Healthy control, EDSS, expanded disability status scale; GGT, gamma-glutamyltransferase; ALT, alanine transaminase; CRP, C-reactive proteins. p: NMO vs MS.

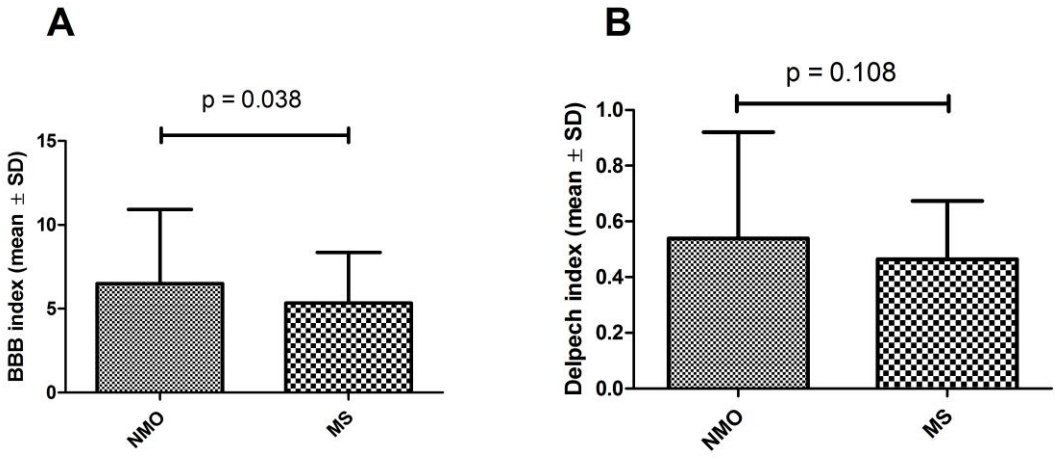
Table 2 Comparison of clinical parameters, serum GGT level, and BBB index, Delpech index in NMO patients with anti-AQP4 antibody seropositive and with anti-AQP4 antibody seronegative

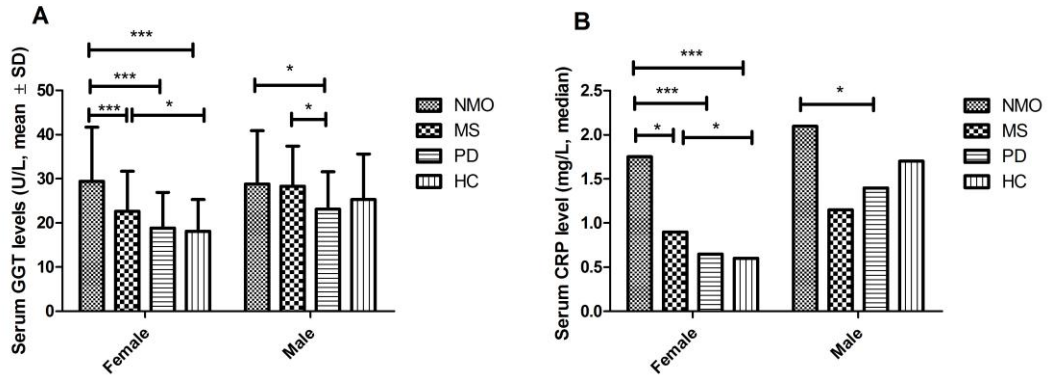
	Anti-AQP4 antibody seropositive (n=82)	Anti-AQP4 antibody seronegative (n=24)	p
Age(years, mean±SD)	38.13 ± 13.39	31.83 ± 13.94	0.057
Disease duration(months, median, range)	24 (0.1 – 240.0)	7.3 (0.1 – 240.0)	0.082
Annualized relapse rate (median, range)	3 (1 - 12)	2 (1 - 7)	0.03
EDSS score (mean±SD)	3.7 ± 2.2	4.0 ± 2.5	0.526
GGT (U/L, mean±SD)	29.37 ± 12.51	28.87 ± 11.22	0.852
CRP (mg/L, median, range)	1.85 (0 – 21.2)	2.00 (0 – 10.3)	0.916

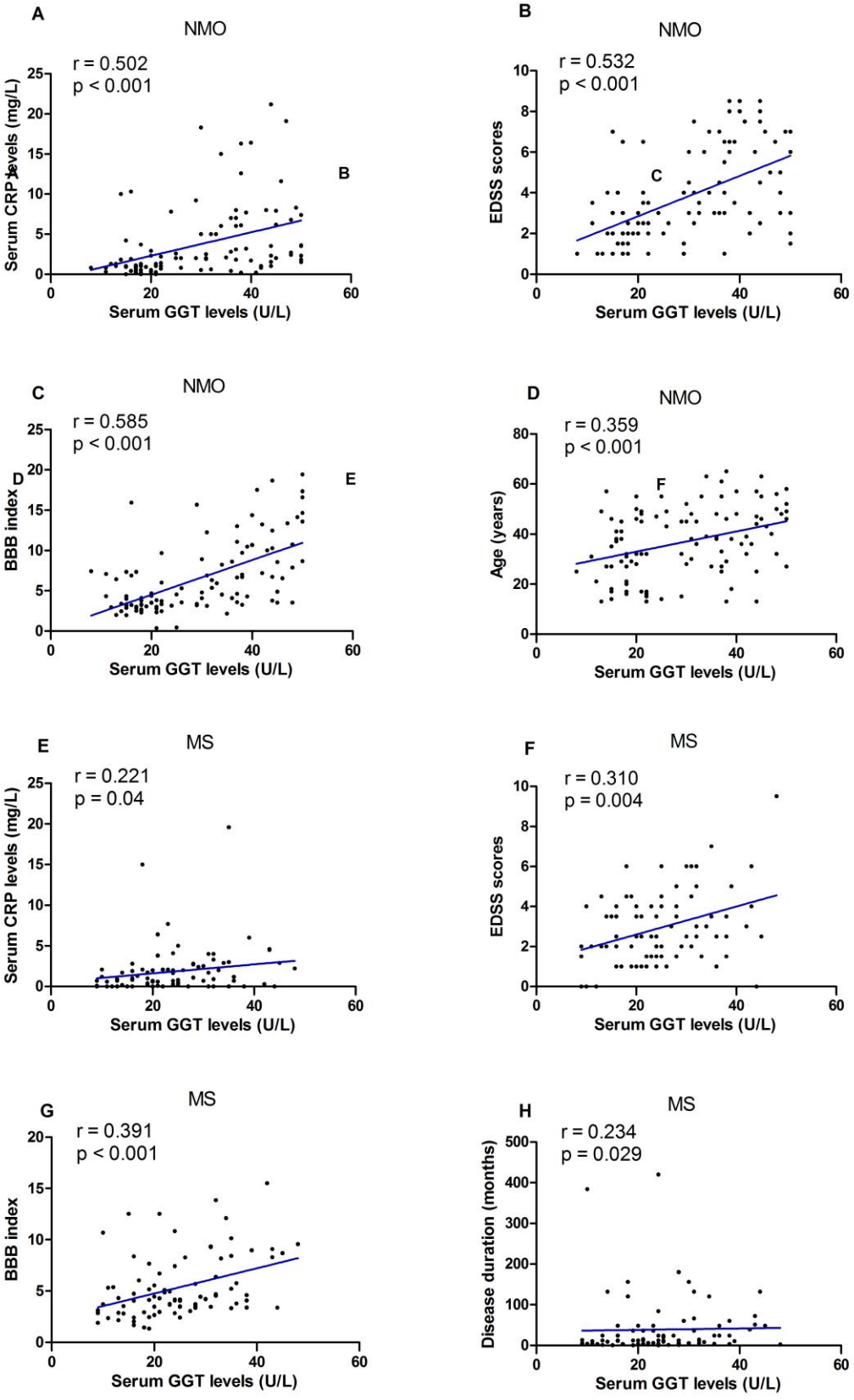
BBB index (median, range)	4.66 (0.37 – 19.43)	4.07 (0.45 – 18.67)	0.514
Delpech index (median, range)	0.47 (0.16 – 0.95)	0.50 (0.14 – 4.0)	0.564

GGT, gamma-glutamyltransferase; CRP, C-reactive protein; NMO, neuromyelitis optica; EDSS, expanded Disability Status Scale; AQP4, aquaporin 4; BBB, blood-brain barrier. SE, standard error.








A
NMO
r = 0.502
p < 0.001
Serum CRP levels (mg/L)
Serum GGT levels (U/L)
B
NMO
r = 0.532
p < 0.001
EDSS scores
Serum GGT levels (U/L)
C
NMO
r = 0.585
p < 0.001
BBB index
Serum GGT levels (U/L)
D
NMO
r = 0.359
p < 0.001
Age (years)
Serum GGT levels (U/L)
E
MS
r = 0.221
p = 0.04
Serum CRP levels (mg/L)
Serum GGT levels (U/L)
F
MS
r = 0.310
p = 0.004
EDSS scores
Serum GGT levels (U/L)
G
MS
r = 0.391
p < 0.001
BBB index
Serum GGT levels (U/L)
H
MS
r = 0.234
p = 0.029
Disease duration (months)
Serum GGT levels (U/L)

Highlights

1. This is the first study to analyze serum GGT levels in NMO and MS patients.

2. Serum GGT and CRP levels were elevated within their normal ranges in NMO and MS patients versus controls.

3. The blood brain-barrier destruction in NMO patients was more severe than that in MS patients.

4. Elevated serum GGT level may be associated with the inflammatory response, oxidative stress and BBB destruction in NMO and MS patients.