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ARTICLE

Serum YKL-40 levels in patients with multiple sclerosis

Níveis séricos de YKL-40 em pacientes com esclerose múltipla

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ABSTRACT

Background: Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system. The YKL-40 protein, which is secreted from various cells that contribute to inflammation and infection, plays a role in immune regulation. **Objective**: This study investigated the serum YKL-40 levels of patients with clinically isolated syndrome (CIS) and MS. **Methods**: The participants was divided into three groups: 1) patients with CIS (n = 20); 2) patients with relapsing-remitting MS (RRMS; n = 39); and 3) healthy individuals (n = 35). The YKL-40 levels in serum samples obtained from the participants were measured using enzyme-linked immunoassays. **Results**: The median serum YKL-40 level was 20.2 ng/mL (range 9.8–75.9 ng/mL) in the patients with CIS, 22.7 ng/mL (range 13.4–57.9 ng/mL) in the patients with RRMS and 11.0 ng/mL (range 10.0–17.3 ng/mL) in the control group ($p < 0.001$). The serum YKL-40 levels in the patients with RRMS were correlated with the patients' expanded disability status scale scores and ages ($p < 0.05$). No relationships were determined between the serum YKL-40 levels and the other variables ($p > 0.05$). The serum YKL-40 levels were higher in the CIS group than in the MS group. These findings show that the serum YKL-40 levels were high even at the beginning of the disease. The serum YKL-40 levels were also not involved in the progression to clinically definite MS. **Conclusions**: The findings from this study suggested that YKL-40 may be a useful marker for the inflammatory process of MS.

Keywords: Multiple Sclerosis; Demyelinating Diseases; Multiple Sclerosis, Relapsing-Remitting; Chitinase-3-Like Protein 1.

RESUMO

Antecedentes: A Esclerose Múltipla (EM) é uma doença inflamatória crônica que afeta o sistema nervoso central. A proteína UKL-40, secretada de várias células que participam de processos inflamatórios e infecciosos, desempenha um importante papel na regulação imunológica. **Objetivo**: Este estudo investigou níveis séricos de YKL-40 em pacientes com Síndrome Clinicamente Isolada (SCI) e EM. **Métodos**: Os participantes foram divididos em três grupos: 1) pacientes com SCI (n = 20); 2) pacientes com EM recorrente-remitente (EMRR; n = 39); e 3) indivíduos saudáveis (n = 35). Os níveis de YKL-40 em amostras séricas obtidas dos participantes foram medidos usando-se imunoensaios ligados a enzimas. **Resultados**: O nível sérico médio de YKL-40 foi 20.2 ng/mL (range 9.8–75.9 ng/mL) em pacientes com CIS, 22.7 ng/mL (intervalo entre 13.4–57.9 ng/mL) em pacientes com EMRR e 11.0 ng/mL (intervalo entre 10.0–17.3 ng/mL) no grupo controle ($p < 0.001$). Os níveis séricos de YKL-40 em pacientes com EMRR estavam correlacionados às pontuações e idades dos pacientes na EDSS ($p < 0.05$). Não foram determinadas relações entre os níveis séricos de YKL-40 e outras variáveis ($p > 0.05$). Os níveis séricos de YKL-40 no grupo SCI estavam mais elevados do que no grupo EM. Estes resultados demonstram que os níveis séricos de YKL-40 estavam mais elevados até mesmo no início da doença. Os níveis séricos de YKL-40 também não estavam associados à progressão da EM clinicamente definida. **Conclusões**: A partir deste estudo, os resultados sugeriram que a proteína YKL-40 pode ser um indicador útil no processo inflamatório da EM.

Palavras-chave: Esclerose Múltipla; Doenças Desmielinizantes; Esclerose Múltipla Recidivante-Remitente; Proteína 1 Semelhante à Quitinase-3.

INTRODUCTION

Multiple sclerosis (MS) is a chronic degenerative disease with clinical symptoms that include axonal injury and loss in the central nervous system[1]. Relapsing-remitting MS (RRMS) is a form of MS that is clinically characterized by exacerbations or relapses. In this disease, autoreactive immune cells migrate into the central nervous system, causing focal inflammation and demyelination. Clinical isolated syndrome (CIS) is another demyelinating disease of the central nervous

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system, and may be the first symptom of MS. The mechanism leading to MS symptoms is not yet known, but may involve YKL-40 (chitinase-3-like protein 1 [CHI3L1]), a glycoprotein that plays a role in occurrences of inflammation, angiogenesis and fibrosis.

YKL-40 is generated by macrophages, neutrophils and cancer cells, and it acts by regulating the levels of vascular endothelial growth factor (VEGF)[2,3]. The human YKL-40 gene is located in a highly maintained area on chromosome 1q31-q32. YKL-40 belongs to family 18 of glycosyl hydrolases, which consists of various types of chitinases[4,5]. High serum levels of YKL-40 have been found in several inflammatory diseases and malignancies[6], and the levels of YKL-40 in cerebrospinal fluid (CSF) are particularly high among people with neurodegenerative and neuroinflammatory diseases[7,8]. Studies in the literature have shown a relationship between YKL-40 and MS, since YKL-40 is predominantly produced by reactive astrocytes in chronic active MS lesions[9].

Very few studies have investigated the serum YKL-40 levels in patients with CIS and MS, although one study reported that the YKL-40 levels were higher in patients with CSF of MS than in healthy individuals. The aim of this study was to evaluate the serum YKL-40 levels of patients with MS.

METHODS

The participants included 59 patients with RRMS (n = 39) and CIS (n = 20) and 35 healthy individuals. The patients were aged between 18 and 65 years and their condition had been diagnosed by an expert neurologist, in accordance with the 2010 McDonald criteria[10].

This study was carried out in the Neurology Department of the Dicle University Faculty of Medicine. The patients with RRMS were treated with IFN-β and glatiramer acetate (GA). Blood samples were taken from the patients with CIS prior to treatment. The expanded disability status scale (EDSS) scores of the patients were obtained during the outpatient follow-up visits[11].

This study was approved by the Clinical Research Ethics Committee of the Faculty of Medicine at Dicle University (ethics approval no: 233). The exclusion criteria of the study were as follows: acute infection, fever, diabetes mellitus, rheumatologic diseases, anemia, kidney dysfunction, autoimmune disease or trauma. All participants in the study were informed about the study prior to giving their consent to participate.

Blood samples were withdrawn into biochemistry tubes. In accordance with the manufacturer's protocol, blood was taken from the patients and control groups and then kept in the laboratory for 15 minutes to facilitate clotting. The blood samples were then centrifuged at 5000 rpm for 10 minutes, transferred to Eppendorf tubes and kept in a freezer at -80 °C until analysis. The YKL-40 levels were determined using the YLbiont brand of commercial ELISA kits and a Biotek-brand ELISA device.

Statistical analysis

Statistical analyses were performed using the SPSS 18.0 software (SPSS Inc., USA). Categorical variables were expressed as numbers and percentages. The Mann-Whitney U test was applied to compare the differences between two independent groups when the dependent variable was either ordinal or continuous. The Kruskal-Wallis test was implemented to compare more than two groups. Spearman correlation analysis was used to determine the relationships between the data. The level of statistical significance was set at 5%.

RESULTS

The mean age of the patients with RRMS (31 females and 8 males) and the patients with CIS (15 females and 5 males) was 30.3 ± 9.25 years, while the mean age of the control group (27 females and 8 males) was 30.2 ± 9.1 years. The mean duration of the disease was 4.7 ± 4.0 years and the mean EDSS score was 1.9 ± 2.0. The mean serum levels of YKL-40 in the patients with RRMS, patients with CIS and healthy individuals were 22.7 pg/ml, 20.2 pg/ml and 11.0 pg/ml, respectively (Table 1).

The serum YKL-40 levels were higher in the patients with RRMS and CIS than in the control group, and this difference was statistically significant ($p < 0.001$). No difference was determined between the two patient groups.

The EDSS scores significantly correlated with the serum YKL-40 levels ($r = 0.372$; $p = 0.20$) ($p < 0.05$). Age also significantly correlated with disease duration in the RRMS group ($r = 0.447$; $p = 0.04$) ($p < 0.01$) (Table 2).

Table 1. Comparison of the YKL-40 levels between the CIS and MS groups.

		Control group (n = 35)	CIS patients (n = 20)	RRMS patients (n = 39)	p
Gender	Female	27	15	31	
	Male	8	5	8	
YKL-40 [pg/mL]	11.0** [10.0-17.3]	20.2** [9.8-75.9]	22.7** [13.4-57.9]	< 0.001	

**Data are expressed as median [Min-Max]. CIS: Clinically isolated syndrome; RRMS: Relapsing-remitting multiple sclerosis; MS: Multiple sclerosis.

Table 2. Correlation coefficients between the parameters in the RRMS group.

		Year	Disease duration	EDSS	YKL-40
Year		1			
Disease duration	r	0.447**	1		
	p	0.004			
EDSS	r	0.244	0.217	1	
	p	0.135	0.185		
YKL-40	r	0.176	0.175	0.372*	1
	p	0.285	0.288	0.020	

*p < 0.05; **p < 0.01. RRMS: Relapsing-remitting multiple sclerosis; EDSS: expanded disability status scale.

DISCUSSION

The findings from the present study demonstrated that the serum levels of the glial marker YKL-40 were higher in patients with CIS and RRMS than in the healthy individuals in the control group. A strong correlation was detected between disease duration, EDSS and age, among the patients with RRMS.

The positive correlations between the YKL-40 levels and age, EDSS and disease duration may be explained by the progression of inflammation. The increased serum YKL-40 levels in the patients with CIS also indicated that the inflammation began intensively even in the first years of the disease. However, elevated YKL-40 levels in the early stages did not indicate progression of the disease.

This study was the first to evaluate the serum YKL-40 levels in patients with RRMS and CIS. YKL-40 levels had previously been evaluated in CSF; for example, Comabella et al.[12] determined that the YKL-40 levels were high in CSF in the first period of MS. These authors also suggested that YKL-40 could be used as a prognostic marker for disability among patients who would progress to clinically definite MS. In the present study, the serum YKL-40 levels were high during the CIS period and were correlated with disability. The YKL-40 levels could also have been affected by treatment, as suggested by Malmeström et al., who found that immunosuppressive treatment decreased the YKL-40 levels in the CSF[13]. The present study also revealed that serum YKL-40 levels were high in patients treated with IFN-β and GA.

In conclusion, serum YKL-40 levels were increased in patients with RRMS and CIS. Additional comprehensive studies should be conducted to further evaluate serum YKL-40 as a biomarker. Another point worth mentioning is that high serum values in patients with CIS may not be related to the transition to clinically definite MS. Further investigations should be conducted to evaluate the use of serum levels of YKL-40 as a biomarker in MS transformation.

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