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Prevalence of Macrocreatinkinase Type 1 in Patients with Inflammatory Bowel Disease

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Abstract Macro-creatine-kinases are isoenzymes of creatinine-kinases (CK). They have been classified in two types: type 1 (CK bound to an immunoglobulin) and type 2 (an oligomeric mitochondrial CK). CK type 1 has been found in patients with ulcerative colitis (UC) but not in Crohn's disease (CD). However, there are no studies evaluating macro-creatinkinase prevalence in inflammatory bowel disease (IBD). We included 159 consecutive patients (72 UC, 85 CD; 2 indeterminate colitis). Creatin-kinase total activity and isoenzymes activities were determined. Twelve (16.7%) patients with UC and one of the two patients with indeterminate colitis had serum macro-creatinkinase type 1 while no CD patients displayed this macromolecule ($P < 0{,}001$). Sensitivity, specificity, positive and negative predictive value, and positive and negative likelihood ratio were calculated for ulcerative colitis versus Crohn's disease diagnosis, being 16.7, 98.9, 92.3, 59, 14.5, and 0.84% respectively. There was no correlation with age, gender, time from diagnosis, associated diseases, concomitant medication or disease activity. In conclusion our data suggests that the presence of macro-CK in IBD favors the diagnosis of ulcerative colitis. Further studies are necessary to understand the significance of this finding in a subset of patients with IBD.

Keywords Inflammatory bowel disease · Ulcerative colitis · Crohn's disease · Diagnostic tests

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Introduction

Creatinine-kinase (CK) is an enzyme used as a marker of ischemic cardiovascular and muscular diseases. There are three CK isoenzymes, which result from the combination of two isomers; CK-MB is the cardiovascular component and plays an important role in differential diagnosis in ischemic cardiovascular diseases, CK-MM is the muscular element, and CK-BB is detected mainly in the brain. A false CK-MB increase with respect to normal total-CK may appear with certain measurement procedures when CK-BB or macroenzymes are present in serum.

Macrocreatinkinase (macro-CK) are CK isoenzymes with a high molecular weight and a different electrophoretic motility compared to the rest of CK isoenzymes. There are two types of macro-CK in the serum [1]. Type 1 is a binding of a CK enzyme and an immunoglobulin (usually IgG or IgA) and is detected in 2% of the population. In early reports it was not associated with specific diseases [1, 2]. However, 49 out of more than 8,000 patients with type I were found more frequently to be women, older individuals, and with complications of cardiovascular diseases; seven of these 49 patients had markers of autoimmune disease [3]. Lee et al. found that more than 50% of patients with macro-CK type I had myositis [4]. Type 2 is a

polymer of mithocondrial CK oligomeric and is released as a result of tissue necrosis [1]. Type 2 has been associated with severe liver disease and malignancy [1, 5, 6].

The relationship between macro-CK and inflammatory bowel disease (IBD) was first described by Tozawa et al. [7], who reported 10 cases of macro-CK type 1 in patients with UC. In 2001 Perez Calle et al. reported three cases of macro-CK type 1 in patients with UC [8]. Although sporadic association of macro-CK and IBD has been described, the true prevalence of this macroenzyme in IBD has not been studied as far as we know. Therefore, we have designed a study to determine the prevalence of macro-CK type I in IBD patients. We also aimed to compare the prevalence of this macromolecule in different IBD subtypes and to study the relationship of macro-CK with age, gender, diseases that can elevate CK-MB and characteristics of disease such as severity and extent, time from onset, and treatment.

Patients and methods

One hundred and fifty-nine consecutive patients diagnosed with IBD attending our IBD unit as in- or outpatients were included. Clinical and treatment data were collected (Table 1). Clinical activity disease index for Crohn's disease (CD) patients and Truelove-Witts index for UC patients were performed. An Hitachi 747 autoanalyser (Roche diagnostics) was used to determine total serum CK by an enzymatic method that measures catalytic activity, the optimized standard method of the Deutsche Gessellschaft für Klinische Chemie [9, 10]. An Hitachi 917 autoanalyser (Roche diagnostics) was used to determine CK-MB isoenzymes activity values by an immuno-inhibition method based on the modified Würzburg assay [11]. Electrophoresis in agarose gel was done in all patients (CK isoenzyme Electrophoresis Kit, P/N 655930) using a ParagonElectrophoresis System (Beckman Instruments Inc., 015-556675-A, 1991). Isoenzymes were identified by ultraviolet light fluorescence and then quantified by fluorometry in a densitometer (Appraise Densitometer System Beckman Instruments Inc., Brea CA).

Table 1 Clinical and treatment features of IBD

	IBD subtype		
	UC (n = 72)	CD (n = 85)	IC (n = 2)
Gender (male/female)	33/39	51/34	1/1
Mean age	46.8 ± 18.6	33.9 ± 12	28.5 ± 16.26
Disease severity			
Inactive (%)	35 (48.6)	29 (34.1)	1 (50)
Mild (%)	16 (22.2)	33 (38.8)	1 (50)
Moderate (%)	17 (23.6)	23 (27.1)	0 (0)
Severe (%)	4 (5.6)	0 (0)	0 (0)
Mean time from onset (months)	86.32	70.22	55
Treatments			
Immunosuppression	8	14	0
Ongoing steroids	14	20	0
Infliximab	0	0	0

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease; IC: indeterminate colitis

Statistical analysis

Results were expressed as mean and standard deviation for quantitative variables and as a percentage with 95% confidence interval (CI) for qualitative variables. The chi-squared (χ^2) test was used to compare percentages and the Student t was used to compare quantitative variables. For all studies, an associated probability (P-value) of <0.05 was considered statistically significant. Sensitivity, specificity, positive and predictive values, and positive and negative likelihood ratios of macro-CK type 1 were calculated for the diagnosis of UC versus CD.

Results

A total of 12 (16.7%, 95%CI = 9.6–27%) UC patients and one (50%, 95%CI = 9.5–91%) indeterminate colitis patient had serum macro-CK type 1. No CD patient presented this macromolecule ($P < 0.001$). One CD patient with bladder neoplasm had type 2 macro-CK. Macro-CK type-1 prevalence in IBD patients was 8.2% (95%CI = 4.7–14%) and 16.7% (95%CI = 9.6–27%) in the UC patients. Sensitivity, specificity, positive predictive value and negative predictive value were 18% (8.1–25%), 99% (97–102%), 92% (78–107%), and 57% (51–67%), respectively. The positive likelihood ratio was 14.5 (95%CI = 1.9–109) and the negative likelihood ratio was 0.84 (95%CI = 0.76–0.94).

There was no relationship between macro-CK and any disease characteristic (Table 2). Patients with immunomodulatory treatment did not present the macromolecule, although this was not statistically significant. No patient with macro-CK had any concomitant disease previously associated with macro-CK (autoimmune diseases other than IBD, neoplasm, cardiocirculatory or musculoskeletal system diseases). CK-MB isoenzyme curve in electrophoresis was higher than the upper normal value (3%) in 38.3% of UC cases and 19% of CD cases ($P = 0.01$). CK total activity was higher than the upper normal value (range 24–195 U/l) in three out of 13 cases with macro-CK type 1. Nine patients had a CK-MB isoenzyme activity value

Table 2 UC patients with and without macro-CK type 1

	Macro-CK+	Macro-CK–	*P*
Mean age (yr)	48.9	46.4	n.s.
Gender			
Male	5	28	n.s.
Female	7	32	
Mean time from onset (months)	89.7	85.7	n.s.
Disease severity			
Inactive	6	29	n.s.
Mild	2	14	
Moderate	2	15	
Severe	1	3	
AZA/6-MP			
Yes	0	8	n.s.
No	11	53	
Disease extent			
Proctitis/proctosigmoiditis	3	14	n.s.
Left-sided colitis	2	20	
Extensive colitis	2	12	
Pancolitis	5	14	

Macro-CK: macrocreatinkinase;AZA: azathioprine; 6MP: 6mercaptopurine; n.s.: not significant

above total CK activity. CK-MB activity was higher than 70% in the other four patients. Mean CK total activity was clearly higher in patients with macromolecules (268 ± 65) than in patients without these molecules (78 ± 140).

Discussion

Macroenzymes are serum enzymes that conform high-molecular-weight complexes by autopolimerization or linkage to another serum element (immunoglobulin). High results from enzyme assays may lead to confusion when the presence of these macromolecules is not suspected. The first macroenzyme discovered, macroamylase, was reported in 1964 [6]. Macroenzymes may be clinically interesting since they are associated with various diseases and sometimes may become diagnostic markers. The prevalence of macro-CK varies from 0.61 to 3.1% [7, 12] depending on the detection technique and the study population. All enzyme-linked immunoglubiln complexes were found in 0.23% in 42,000 patients selected at random and 10,000 blood donors [7].

Macro-CK type 1 is a binding of one or two CK molecules (usually CK-BB isoenzyme) to the Fab region of an immunoglobulin (usually IgG with Kappa light chain type), which moves between CK-MB and CK-MM in the electrophoretic spectrum (Fig. 1). A macromolecule or an interference is usually suspected when CK-MB activity

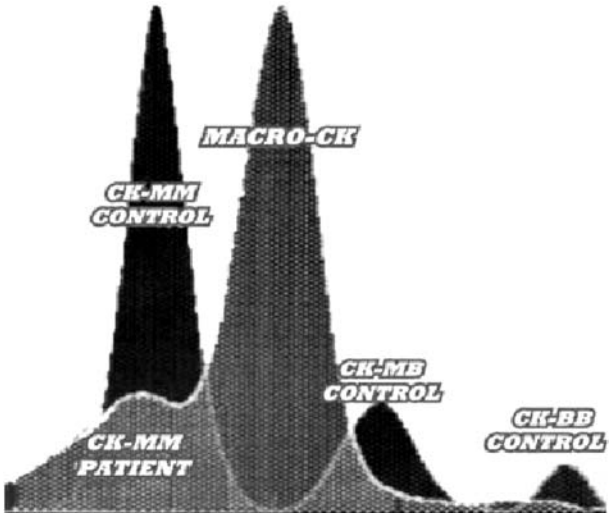

Fig. 1 Electrophoretic pattern of macro-CK type 1 compare with control. Macro-CK: macrocreatinkinase. CK-MM: isoenzyme MM of creatinkinase. CK-MB: isoenzyme MB of creatinkinase. CK-BB: isoenzyme BB of creatinkinase

(immuno-inhibition method) accounts for more than 15 to 20% of total CK activity since higher values are not observed in myocardial infarction [1, 13]. However, macroenzymes may appear with even lower values. In our study CK-MB activity was always above 70% of total CK activity. No relation between macro-CK and a specific pathology has been described, although it has been associated with cancer, hypothyroidism, myositis, cardiovascular events, autoimmune diseases, etc. [3, 4, 6]. A high association of macro-CK with autoimmune diseases was described [7]. There were 10 cases with UC but no mention of any CD patient in this study was made.

The presence of this macroenzyme in selected IBD patients has not been evaluated. The prevalence of macro-CK type 1 in our IBD study was 8.2%. Although our study lack of a healthy control group, this prevalence is clearly above the overall prevalence reported previously in blood donors and hospitalized patients. The macromolecule was only associated with UC or indeterminate colitis but not with CD.

Macro-CK type 1 in UC probably reflects the presence in serum of antibodies against CK molecules. Overall prevalence of autoantibodies in UC is 30% and they are not considered to be pathogens but reactive antibodies [14]. The most important autoantibody, pANCA, has a mean prevalence of 55% for UC and 17% for CD [15].

We did not find any feature associated with this molecule in UC patients; for this reason its determination will not be useful in assessing treatment efficacy or extension of inflammation. The low prevalence and low sensitivity of macro-CK type-1 limit its clinical utility, but the high specificity of this test for detecting UC patients if confirmed

in larger series of patients could help to differentiate UC and CD. Furthermore, the high value of the positive likelihood ratio for macro-CK in UC in our study indicates a high probability of UC diagnosis in one IBD patient with this macromolecule.

Further studies are needed to confirm the role of macro-CK type 1 in IBD diagnosis. Nevertheless, it is important to be aware of the association with UC and IBD in order to avoid misdiagnosis when macro-CK and thoracic pain co-occur in a IBD patient.

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