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Review

Laboratory and clinical features of abnormal macroenzymes found in human sera ☆

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ABSTRACT

We report the analysis of unusual macroenzymes, performed in our laboratory, and review the relevant literature. In particular, we focused on macro AST, macroamylase, macro LD and macro CK. Macroenzymes are seen in healthy subjects, but can also be related to disease; thus, accurate detection is useful in day-to-day clinical practice. The macroenzyme is thought to be a specific antigen–antibody complex from the following findings: (1) the complex could be dissociated under acidic pH levels; (2) binding specificity of immunoglobulin in the complex was observed; (3) the binding site of immunoglobulin in the complex was Fab portion; and (4) the maternal IgG involved with macroenzyme was transferred to her children. This article is part of a Special Issue entitled: Medical Proteomics.

1. Introduction

Numerous patient serum samples are tested in biochemical laboratories every day. Measurement of serum enzyme activity is one of the most common tests, and is performed using computerized, automated analyzers. Although automated analyzers have contributed greatly to mass analysis in clinical laboratories, there is an increasing tendency to depend on them. Consequently, clinicians often fail to evaluate the individual data printed out by these analyzers.

The so-called spurious or factitious data are certainly present among the enormous amounts of raw data obtained by analyzers, and these data are not derived from mechanical and/or systematic errors. A typical example of these results is abnormal high-mass molecular enzyme, so-called macroenzymes, found in human sera. Macroenzymes are formed by association with serum immunoglobulins. At present, numerous macroenzymes are known, for example, macro aspartate aminotransferase [EC 2.6.1.1; AST] [1,2], macroamylase [EC 3.2.1.1] [3,4], macro lactate dehydrogenase [EC 1.1.1.27; LD] [5,6] and macro creatine kinase [EC 2.7.3.2; CK] [7,8]. The existence of these macroenzymes has long been known, but they have only recently become a problem in clinical practice. Here, we describe our analyses of abnormal macroenzymes in our laboratory, and review the available data from previous macroenzyme studies. It is important for clinical laboratories to change the characterization of macroenzymes and their laboratory detection methods in order to avoid errors.

2. History of macroenzymes

In 1967, Berk et al. [3] reported the existence of macromolecular amylase in sera from patients with persistently elevated serum amylase. In these patients, there was no apparent cause for hyperamylasemia, and their renal function was essentially normal. This was the first description of a so-called "macroenzyme". In 1968, Levitt and Cooperband [9] confirmed IgA as a binding protein in the abnormal macromolecular amylase in patient serum.

Abbreviations: EC, Enzyme Commission; AST, Aspartate aminotransferase; sAST, Supernatant aspartate aminotransferase; mAST, Mitochondrial AST; Camy, Amylase clearance; Ccr, Creatinine clearance; ALT, Alanine aminotransferase; LD, Lactate dehydrogenase; ALP, Alkaline phosphatase; GGT, gamma-glutamyltransferase; LAP, Leucine aminotransferase; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; Fab, Fab fragment of immunoglobulin molecule; Fc, $F(ab')_2$, $F(ab')_2$ fragment of immunoglobulin molecule; Fc, fragment of immunoglobulin molecule; HPLC, High performance liquid chromatography

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In 1965, Kreutzer [10] reported an anomalous electrophoretic pattern for lactate dehydrogenase (LD) isoenzymes. In 1967, Ganrot [5] reported macromolecular LD in serum from a patient with lupoid cirrhosis and an anomalous electrophoretic isoenzymic pattern, and identified IgA as the binding protein in the complex. The factors leading to the discovery of macroamylase and macro LD were high serum activity and/or unusual patterns on electrophoretic isoenzymic analysis.

In the 1970s, macro alkaline phosphatase [EC 3.1.3.1] was reported by Nagamine et al. [11], macro aspartate aminotransferase [EC 2.6.1.1; AST] was reported by Konttinen et al. [1], alanine aminotransferase [EC 2.6.1.2; ALT] was reported by Kajita et al. [12], and macro creatine kinase [EC 2.7.3.2; CK] was reported by Urdal et al. [7]. Moreover, in the 1980s, γ-glutamyltransferase [EC 2.3.2.2] was reported by Sudo et al. [13], acid phosphatase [EC 3.1.3.2] was reported by Virji [14], leucine aminopeptitase [EC 3.4.11.2] was reported by Maekawa et al. [15], and lipase [EC 3.1.1.3] was reported by Stein et al. [16]. In fact, Maekawa et al. [15] demonstrated various macroenzymes, including macro leucine aminopeptitase, in serum from a patient with rheumatoid arthritis. The author also reported unique and very interesting cases with mitochondrial AST–IgG complex [17] and with salivary amylase–IgG complex [18]. In 1989, Tozawa [19] reported the incidence of macroamylase and macro LD in patients and in blood donors; macroamylase was present in 0.18% and 0.04%, respectively, and macro LD was detected in 0.32% and 0.15%, respectively.

In 1990, we reported the incidence of macro AST in patients with high serum aspartate to alanine aminotransferase ratio (>2.0) using clinical laboratory data [20]; the incidence was 13.1%. It should be noted that macro enzymes are detected in infants and/or children [21–23], and we recently reported transient macro AST in infancy caused by maternal IgG [24]. To date, there have also been several excellent reviews [21,25–31].

3. Macro aspartate aminotransferase (AST)

3.1. Molecular forms of AST and macro AST

AST has two isoenzymes, supernatant or cytosolic AST (sAST or cAST) and mitochondrial AST (mAST), which migrate to the α_2-globulin region and to the slow γ-globulin region on electrophoresis, respectively [17,32]. The molecular mass of each isoenzyme is approximately 100,000. Furthermore, these isoenzymes are immunologically independent, and immunochemical cross-reactivity has been reported between the isoenzymes in mammals [32–34]. Utilizing these properties, in 1978, Teranishi et al. [35] established an immunological method for determination of mAST activity using an anti-pig-sAST antibody. In 1983, Sakakibara et al. [36] developed a convenient staining method for electrophoresis of serum AST isoenzymes. AST isoenzyme analysis is important in the diagnosis of liver disorders and myocardial infarction, and determination of mAST is particularly useful in evaluating the severity of liver damage and myocardial damage [37].

In 1978, Konttinen et al. [1] reported, for the first time, macro AST in sera from two healthy women with an unexplained persistent elevation of AST activity. Electrophoresis of serum AST isoenzymes displayed an abnormally moving fraction, which migrated between the sAST and mAST positions. One of these two cases was confirmed to be an IgG complexed-AST. Similar cases were also reported by Nakajima et al. [38] in 1980 and Weidner et al. [39] in 1983. Nakajima et al. [38] confirmed that macro AST consists of IgG and sAST isoenzymes. In 1983, Nagamine and Okochi [40] reported that a macro AST of IgG, IgA–κ, λ was present in serum from a patient suffering from lung cancer with metastasis to the liver. They demonstrated that the IgG in the complex binds to sAST, whereas IgA binds to both aAST and mAST isoenzymes [40]. In 1986, Moriyama et al. [17] reported a macro AST of mAST linked to IgG in serum from a patient with benign hypertension. We demonstrated that macro AST is a specific antigen–antibody complex using papain digestion of the IgG in the complex. There have since been a number of papers published describing macro AST [2,20,22–24,31,41–70].

3.2. Characterization of our cases of macro ASTs

Table 1 shows a summary of the classes and types of immunoglobulins in macro ASTs identified to date in our laboratory, together with those in other macroenzymes. Fifty-four cases with macro AST have been studied to date. The bound immunoglobulins were confirmed as IgA in 32 cases (59.2%), IgG in 14 cases (25.9%), IgG–IgA in 7 cases (13.0%), and not determined in one case. IgA-type macro AST was found most frequently, but the difference was not significant. The average age at discovery was 44.1 years, ranging from 3 days [24] to 84 years (standard deviation, 26.5 years). Thirty cases were male and 24 were female. Total serum AST activity in each of these cases was above the upper value of the normal range (30 U/l), ranging from 51 to 1408 U/l, except in one mother-to-child case [24] (27 U/l). The mean and standard deviation were 377 and 327 U/l, respectively. The mean and standard deviation of the ratio of AST to ALT were 9.1 and 14.7, respectively, ranging from 1.3 to 70.7. In reconstitution studies ($n = 10$), one case (IgG-κ, λ) was reconstituted with mAST [17], one case (IgG-κ) was reconstituted with sAST and mAST [24], and 8 cases (2 cases of IgA-λ and IgG, IgA-κ, λ, 3 cases of IgG-κ, and 1 case of IgG, IgA-λ) were reconstituted with sAST.

In all cases, one or two macromolecular AST peaks were confirmed by gel permeation chromatography. The amount of free-AST-molecule was observed to vary according to those cases. Typical isoenzyme electrophoretic patterns with macro AST are shown in Fig. 1A. All abnormal bands of IgG complexed-AST showed migration between the sAST and mAST band, while that of IgA complexed-AST showed slightly cathodal migration to the sAST position. However, in the majority of the patients with IgA complexed-AST, the abnormal bands were seen faintly or not detected on ordinary AST isoenzyme electrophoresis [20]. We believe that these abnormal electrophoretic patterns indicate the type, amount and isoenzyme specificity of bound immunoglobulin, from the above studies.

In Table 2, morbidities of cases with macro AST for diseases classified according to the international classification of disease [71] are shown, together with those for other macroenzymes. In 54 patients with macro AST, neoplasms (35.2%) including primary and metastatic liver cancer, colon cancer, pancreatic cancer and malignant lymphoma were most common. Diseases of the digestive system (22.2%) including liver cirrhosis, cholelithiasis and chronic hepatitis were second in frequency. The rest of the cases were patients with circulatory diseases

Table 1
Classes and types of immunoglobulins in macroenzymes.

Macroenzume	Class	κ	λ	κ, λ	ND	Total (%)
Macro AST	G	10	1	3	0	14 (25.9)
	A	0	31	1	0	32 (59.2)
	G, A	0	4	3	0	7 (13.0)
	ND	0	1	0	0	1 (1.9)
	Total	10 (18.5)	37 (68.5)	7 (13.0)	0	54 (100)
Macroamylase	G	6	1	0	0	7 (14.9)
	A	14	19	6	0	39 (82.9)*
	G, A	1	0	0	0	1 (2.2)
	Total	21 (46.8)	20 (42.5)	6 (12.7)	0	47 (100)
Macro LD	G	8	3	7	1	19 (51.3)
	A	11	3	1	1	16 (43.2)
	G, A	0	0	1	1	2 (5.5)
	Total	19 (40.2)	6 (16.2)	9 (24.3)	3 (19.3)	37 (100)
Macro CK type-1	G	2	2	3	0	7 (21.9)
	A	0	11	5	2	18 (56.3)
	G, A	0	1	4	0	5 (15.6)
	ND	0	0	0	2	2 (6.3)
	Total	2 (6.2)	14 (43.8)	12 (37.5)	4 (12.5)	32 (100)

A, immunoglobulin A; G, immunoglobulin G; ND, not-determined.
* $p < 0.005$ (IgA-type vs IgG-type).

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