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Relation between Rheumatoid Arthritis and Alkaline Phosphatase Isoenzymes

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Summary. **Serum biliary ALP isoenzyme (ALP_1) was detected in 29.7% of rheumatoid arthritis (RA) patients studied, whereas it was undetectable in osteo-arthrosis patients. The positive activity of ALP_1 in the serum was accompanied by significant but mild increases in γ-GTP and LAP. These findings suggest that RA has an influence on the biliary tract. Furthermore, elevations of ALP_2, ALP_3, RAHA, CRP, ESR and IgA as well as a lowering of grip strength, stage and class in the ALP_1-positive RA patients were more marked than in the ALP_1-negative RA patients. These data suggest that the disease activity of RA is higher, and the bone resorption mechanism more enhanced in ALP_1-positive than in ALP_1-negative RA patients. There was no significant relationship between ALP_1 and AMA or ANA. This suggests that the possible overlapping of RA and PBC or SLE could be excluded from the reasons of the detection of ALP_1 activity.**

INTRODUCTION

Abnormal elevations of alkaline phosphatase (ALP) serum activity in some rheumatoid arthritis (RA) patients have been reported by Frank and Klemmayer,[1] Kendall et al.,[2] and Perlik and Kutova.[3] Since the development of a method for measuring ALP isoenzymes[4] originating in the biliary tract, liver, bone, placenta and the intestine, a number of findings concerning the relation between RA and ALP isoenzymes have been reported by several investigators.[5-8] These workers found elevations of liver (ALP_2) and/or bone ALP (ALP_3) isoenzymes in the serum of RA patients. However, how these ALP isoenzymes are related to RA remains unknown.

ALP_2 and ALP_3 can always be observed in normal serum, and intestinal ALP isoenzyme (ALP_5) can be detected in the serum of blood types B and O in normal physiological conditions. In contrast, biliary ALP isoenzyme (ALP_1) is usually only detectable in the serum of patients with biliary tract disorders.[4] Rosalki et al.[6] reported 4 RA cases in which ALP_1 activity was detected. Therefore, the present study was carried out to confirm whether ALP_1 activity is detectable in the serum of RA patients and to determine how ALP isoenzyme activities are related to RA by observing total ALP (ALPt), ALP isoenzymes, γ-glutamyl transpeptidase (γ-GTP), leucine aminopeptidace (LAP) and transaminases (GOT and GPT). Furthermore, the relation between these isoenzymes and disease activity of RA was examined by measuring C reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid arthritis hemagglutination (RAHA) and immunoglobulins (IgG, IgA and IgM). Moreover, antimitochondrial antibody (AMA) and antinuclear antibody (ANA) titres were measured in RA patients to determine whether ALP_1 activity results from RA itself or is due to an overlapping of RA and liver involvement from other connective tissue diseases such as primary biliary cirrhosis (PBC) or systemic lupus erythematosus (SLE).

MATERIALS AND METHODS

The subjects in this study were 286 patients with RA (females; 225, males; 61, mean age; 55 ± 12 years)[9] and 212 patients with osteo-arthritis (OA) (females; 148, males; 64, mean age; 61 ± 12 years). Stages and classes of RA patients were determined according to the criteria by Steinblocker et al.[10] These patients had no other diagnosed disorders at the times of this study such as hepatic, biliary, pancreatic, skeletal, intestinal and/or uterine diseases. All RA patients had been treated for more than one month with drugs such as non-steroidal anti-inflammatory drugs

(NSAIDs), corticosteroids and/or disease-modifying anti-rheumatic drugs (DMARDs), and many of the OA patients had been treated with NSAIDs for more than one month.

Laboratory data measured[11] included the following: ALPt (method of Bessy and Lowry), ALP isoenzymes (electrophoresis in cellulose acetate membranes), γ-GTP (γ-glutamyl-p-nitroanilide method), LAP (modified method of Tuppy), GOT (International Federation of Clinical Chemistry method), GPT (International Federation of Clinical Chemistry method), CRP (immunonephelometry), ESR (Westergren method), RAHA (particle antibody method), immunoglobulins (single radial immunodiffusion). AMA (fluorescent antibody technique) and ANA (fluorescent antibody technique).

The results were expressed as the mean and standard deviation (mean $\pm$SD). Date were analyzed statistically using the chi-squared test, analysis of variance (ANOVA) or Wilcoxon's signed rank test. Differences at $p<0.05$ were considered significant.

RESULTS

Serum ALP_1 activity was detected in 85 of 286 RA patients (29.7%), and this activity varied from 4 to 262 U/l (Table 1). In contrast, no ALP_1 activity was detected in 212 OA patients irrespective of whether they were currently being treated with NSAIDs (33.4%) or untreated (66.6%) (6 OA and 2 RA patients who showed positive serum ALP_1 activity had been excluded because the activity was attributable to another disorder such as cholelithiasis). In RA patients, no significant difference was noted in the incidence of positive serum ALP_1 activity irrespective of whether patients were being treated with drugs, such as NSAIDs, corticosteroids and/or DMARDs, or untreated (Table 2).

A significant elevation of ALP_2 was noted in both ALP_1-positive and negative RA patients as compared with OA patients ($p<0.01$), and this ALP_2 activity was significantly higher in ALP_1-positive RA patients than in ALP_1-negative RA patients ($p<0.05$). ALP_3 activity was significantly elevated in only ALP_1-positive RA patients, as compared with ALP_1-negative RA ($p<0.05$) and OA ($p<0.05$) patients. In both types of RA and OA patients, a normal physiological level[4] of ALP_5 was seen in the serum of blood types B or O (20 ± 9 U/l in 32.3% of RA patients, and 18 ± 8 U/l in 34.8% of OA patients), as was also observed by Siede et al.[7] Other ALP isoenzymes such as placental ALP (ALP_4) were not demonstrated in any of our subjects. Mean ALPt activity in both types of RA patients was significantly elevated compared with OA patients ($p<0.01$), and this activity in ALP_1-positive RA patients was significantly higher than in ALP_1-negative RA patients ($p<0.05$) (Table 1). In all RA patients, significant elevations of the activity of ALP_2 (135 ± 147 U/l, $p<0.01$), ALP_3 (75 ± 65 U/l, $p<0.05$) and ALPt (211 ± 190 U/l, $p<0.01$) were seen as compared with OA patients.

In ALP_1-negative RA and OA patients, values of γ-GTP, LAP, GOT and GPT were all within normal limits. There were no significant differences in serum GOT and GPT levels among both types of RA patients and OA patients. There were no clinical symptoms suggesting biliary tract disease in any of the subjects. A significant but mild elevation of γ-GTP and LAP was noted in ALP_1-positive RA patients as compared with ALP_1-negative RA ($p<0.05$) and OA ($p<0.01$) patients (Table 1).

In ALP_1-positive RA patients, CRP ($p<0.01$) and ESR ($P<0.01$) levels (Table 3) as well as RAHA titre ($p<0.01$) (Table 4) were significantly higher, and the lowering of grip strength ($p<0.01$), stage ($p<0.01$) and class ($p<0.01$) were significantly more severe than in ALP_1-negative RA patients (Table 3, Fig. 1). The IgA level in ALP_1-positive RA patients was significantly higher than in ALP_1-negative RA patients ($p<0.01$), while no significantly differences were noted in either IgG or IgM levels (Table 3).

Table 1. Comparisons of serum ALP_1, ALP_2, ALP_3, ALPt, γ-GTP, LAP, GOT and GPT in RA and OA patients.

Patients	N	ALP_1(U/l)	ALP_2(U/l)	ALP_3(U/l)	ALPt(U/l)	γ-GTP(U)	LAP(U)	GOT (U)	GPT (U)
ALP_1-positive RA	85	33 ± 90.0	174 ± 208**#	93 ± 91*#	266 ± 324**#	44 ± 81**#	203 ± 127**#	17 ± 8	15 ± 10
ALP_1-negative RA	201	—	104 ± 34**	64 ± 24	171 ± 57**	14 ± 6	140 ± 25	16 ± 6	14 ± 13
OA	212	—	79 ± 29	62 ± 22	147 ± 44	18 ± 20	147 ± 25	19 ± 8	15 ± 9

*$p<0.05$ and **$p<0.01$ compared with OA, #$p<0.05$ and ##$p<0.01$ compared with ALP_1-negative RA by ANOVA. Values are given as means$\pm$SD.

Table 2. Incidences of serum ALP_1-positive RA patients and their relation to current treatment with NSAID, corticosteroid and/or DMARD.

Treatment		ALP_1		
		positive	negative	Total
NSAID	treated	80(30.0)	187(70.0)	267(100)
	untreated	5(26.3)	14(73.7)	19(100)
Corticosteroid	treated	24(35.8)	43(64.2)	67(100)
	untreated	61(27.9)	158(72.1)	219(100)
DMARD	treated	30(24.2)	94(75.8)	124(100)
	untreated	55(34.0)	107(66.0)	162(100)
Total RA patients		85(29.7)	201(70.3)	286(100)

No significant difference indicated between NSAID, corticosteroid and DMARD treatments by chi-squared tests. Numbers in parentheses represent percentages.

Table 3. Comparisons of CRP, ESR, IgG, IgA, IgM and grasping strength between ALP_1-positive and negative RA patients.

Patients	N	CRP** (mg/l)	ESR** (mm/h)	IgG (mg/dl)	IgA** (mg/dl)	IgM (mg/dl)	Grasping Power(mmHg)	
							left **	right **
ALP_1-positive RA	85	43±38	76±36	2061±625	505±162	269±108	96± 89	84± 91
ALP_1-negative RA	201	23±29	48±33	1984±540	412±162	269± 90	156±122	138±111

**$p<0.01$ by ANOVA. Values are given as means±SD.

AMA was positive (more than 80/1) in 4 out of 199 RA patients (2.0%), and ANA was positive (more than 40/1) in 38 cases (19.1%); one was later diagnosed as overlapping of RA with SLE. However, no significant relationship between AMA or ANA and ALP_1 activity was found (Table 4).

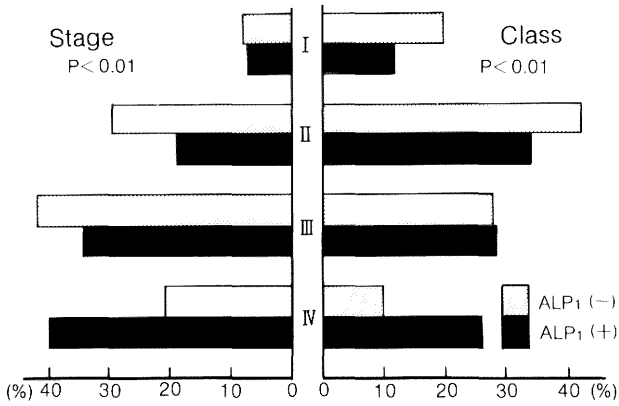

Fig. 1. Comparisons of stage and class between ALP_1-positive and negative RA patients. Wilcoxon's signed rank test was used.

DISCUSSION

From observations of 5-nucleotidase and liver specimens, Kendall et al.[2] speculated that the increased ALPt seen in RA patients was of hepatic origin. Spooner et al.[5] and Rosalki et al.[6] also reached the same conclusion from observations of increases in ALP_2 and γ-GTP. Siede et al.[7] indicated a rise of ALP_3 activity in RA patients. In this study, significant elevations of ALP_2 as well as ALPt activity were noted in both ALP_1-positive and negative RA patients. Therefore, the increase of ALPt in RA patients may be due mainly to the elevation of ALP_2 activity. The higher ALPt activity seen in ALP_1-positive RA patients may be attributed to ALP_1 activity and an increase in ALP_3 activity in addition to the larger increase in ALP_2 activity (Table 1).

One important feature may be the very high incidence (29.7%) of ALP_1 activity that was detected in RA patients. ALP_1 activity is usually found in the serum of patients with biliary tract disorders.[4] The presence of ALP_1 activity in the serum of RA

Table 4. RAHA, AMA and ANA in ALP_1-positive and negative RA patients.

	Patients	N		Titre (/1)								
				≦20	40	80	160	320	640	1280	2560	5120≦
RAHA**	ALP_1-positive RA	85	(%)	8.6	7.1	10.0	14.3	17.1	20.0	7.2	10.0	5.7
	ALP_1-negative RA	201	(%)	21.2	11.8	15.3	18.8	10.6	18.2	2.3	0.6	1.2
AMA	ALP_1-positive RA	59	(%)	98.3(≦40)		1.7	—	—	—	—	—	—
	ALP_1-negative RA	140	(%)	98.6(≦40)		0.7	—	—	0.7	—	—	—
ANA	ALP_1-positive RA	59	(%)	76.3	3.4	13.6	3.4	1.7	—	1.7	—	—
	ALP_1-negative RA	140	(%)	83.6	5.0	5.0	0.7	2.9	0.7	—	2.1	—

**$p<0.01$ within the group by Wilcoxon's signed rank test.

patients was independent of treatment with NSAIDs, corticosteroids and/or DMARDs (Table 2). Therefore, this ALP_1 activity is not attributable to these drugs but to RA itself. Further, this study of 286 RA patients also demonstrated that the presence of ALP_1 activity was associated with increases in ALP_2, γ-GTP and LAP levels (Table 1), whereas no relation was found between ALP_1 activity and the level of GOT or GPT. Serum ALP_2 activity usually rises in parallel with ALP.[4] Considering these facts collectively, it is possible to conclude that the influence of RA on the biliary tract may lead to the serum positive activity of ALP_1.

Frank and Klemmayer[1] suggested that the serum ALPt level in RA patients was usually within normal limits, but was elevated in the active disease state. Kendall et al.[2] mentioned that the disease activity of RA is related to the level of ALPt. Furthermore, other authors have speculated that bone resorption in RA is responsible for increases in ALPt[3] or ALP_3[7] activity. This study found that ALP_3 activity in ALP_1-negative RA and OA patients remained within the normal ranges, whereas in ALP_1-positive RA patients the ALP_3 activity was significantly higher as compared with both ALP_1-negative RA and OA patients (Table 1). This suggests a greater increase in bone resorption in ALP_1-positive RA patients. We also demonstrated that increases in CRP, ESR, IgA and RAHA were higher in ALP_1-positive RA patients than in ALP_1-negative RA patients, and that the lowering of grip strength, stage and class were more severe (Tables 3, 4, Fig. 1). Based on these results, it can be concluded that positive ALP_1 activity is closely related to disease activity in RA. Moreover, in ALP_1-positive RA patients, ALP_2 and ALP_3 activity was also higher than in ALP_1-negative RA patients. Accordingly, measurement of ALP_1 should be considerd as one of the more important parameters for determining disease activity in RA.

Additionally, this study revealed a significantly higher IgA level in ALP_1-positive RA patients (Table 3). These facts suggest that ALP_1 activity becomes detectable in serum when mechanism of IgA production in RA is enhanced, and as a consequence, a greater increase in serum IgA leads to greater RA severity. However, as a significant correlation has not been established, the presence of ALP_1 activity in the serum cannot be directly linked to a mechanism that stimulated IgA production.

Spooner et al.[5] and Kantharia and Woolf[9] assumed a connection between RA and PBC because of the increases in ALP_2 and γ-GTP. There is a possibility that increases in these measurements are brought about by an overlapping of RA and other connective tissue diseases such as PBC or SLE. According to Davis,[12] ANA is usually detectable in sera of not only SLE patients but also more than 20% of RA patients, and in this study no significant relation was found between ALP_1 and AMA or ANA (Table 4). From these facts, the ALP_1 activity observed in RA patients to be attributable to RA itself and not to some secondary cause arising from some other connective tissue disease.

Conclusions about the relation between ALP_1 and RA in previous reports[6-8] were drawn from a very small number of cases. To determine the validity of such conclusions, a larger number of cases must be studied. However, this study has shown that ALP_1 activity was detectable in the serum of 29.7% of RA patients observed, that ALP_2 activity is detectable in all RA patients, and that ALP_3 activity rises in parallel with the serum positive activity of ALP_1. In addition, the disease activity was enhanced in ALP_1-positive RA patients. Thus, it is suggested that measurement of ALP_1 is an important parameter in determinig disease activity in RA.

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