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≪Original Article≫

Serum Alkaline Phosphatase : Age and Sex Differences of Values in Adolescents and its Isoenzyme Characteristics

NAHOKO FUKUDA and KIYOH TANISHIMA

Department of Medical Technology, Paramedical School of Kanazawa University,
5—11—80 Kodatsuno, Kanazawa, Japan 920

SUMMARY Serum alkaline phosphatase levels in normal adolescents were studied in 247 males and 237 females subdivided by sex, age and blood type. Isoenzyme characteristics in some cases were also studied.

Alkaline phosphatase activity was measured by the kinetic method for sera from 10, 13, 16 and 19 years old men and women. No statistically significant influence of blood type on enzyme activity was observed in all cases. Alkaline phosphatase level at age 19 is essentially equal to that of adults and shows no significant difference between males and females. The activities in both sexes at age 10, 13 and 16 are in apparent higher level, and the difference in the activities between males and females are observed at these ages. From the study of isoenzyme characteristics, it is considered that the high level of alkaline phosphatase in the serum from adolescent males and females is due to the high bone phosphatase seen during growth.

The variations in those normal levels and isoenzyme characteristics are of clear importance in assessing data from individual adolescent patients, for example, in comparison to adolescent with obesty.

Introduction

There are many variables which are known to influence serum alkaline phosphatase activity[1)-6)]. Particular changes in distribution of normal values exist in adolescence with regard to age, sex and blood type, and it is not proved whether isoenzymes of bone, liver and intestine alkaline phosphatase contribute to this changes in distribution.

This makes it difficult particularly to interpret the laboratoy tests from young patients with hereditary and acpuired disordes of bone growth and development. The purpose of this paper is to report the precise level of serum alkaline phosphatase activity with age, sex and blood type in healthy adolescent Japanese as measured by a newly accepted kinetic method under attentive quality-control, and to report the contribution of alkaline phosphatase isoenzymes by bone, liver and intestine.

金沢大学医療技術短期大学部衛生技術学科
(〒920 金沢市小立野5丁目11—80)
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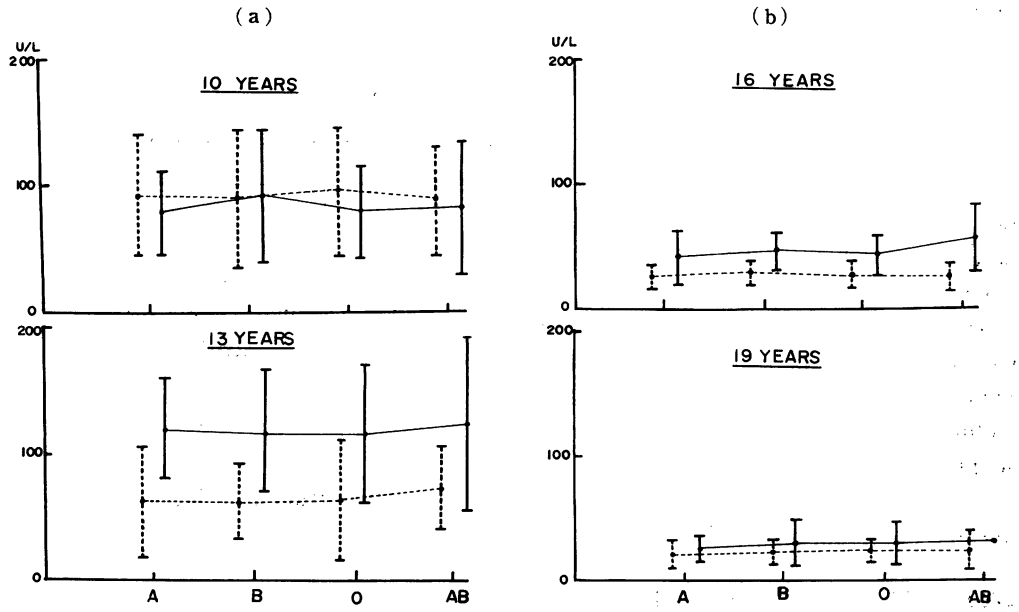
Materials and Methods

Serum samples were obtained from healthy schoolchildren and students who were invited to volunteer for the project in September, 1977 on the survey of the influence of sport on their growth by

Japan Amateur Sport Association. From eight schools and one college in Kanazawa City, 247 males and 237 females who were in a cross-sectional socioeconomic backgrownds participated in the survey. Their ages were 10, 13, 16 and 19 years. All the blood samples were obtained from ante-cubital veins between 1 : 00 p. m. and 3 : 00 p. m. Serum was separated within 2 hours of collection by means of Sure-Sep separator (General Diagnostics), allowed to refrigerate at 4°C and was analyzed the following day. All hemolyzed and lipemic samples were rejected. The simultaneous screening of the subjects included height, weight and skinfold measurements, a physical examination, multiple blood pressure readings, electrocardiogram, psychologic assessment, full clinical examination, and hematological examination. The subjects with any of the abnormalities were excluded.

Alkaline phosphatase activity was estimated by our kinetic method[7] modified from that of Bowers and McComb. The substrate mixture had the following composition (in m moles/liter) : p-nitrophenylphosphate (10), 2-amino-2-methyl-1, 3-propandiol buffer pH 10.5 (50), and $MgCl_2$ (0.5). The reaction was started by adding 0.1 m*l* of the sample to 2.0 m*l* of the substrate mixture, and the initial rates of reactions were measured with a Hitachi Model 200 recording spectrophotometer at 25°C, the absorbance at 404 nm being recorded for at least 2 min. The enzyme activities were expressed in IUB units (U) per liter. The millimolar absorptivity (liter m mol^{-1}. cm^{-1}) of 18.75 was used for p-nitrophenol. As control sera was used Moni-Trol I (DADE Co.) dissolved in 20 g/d*l* glycerol which was improved in our laboratory[7].

DISC-electrophoresis on polyacrylamide



Bars indicate ±2 S. D. of mean. Solid lines : male. Dotted lines : female.
The enzyme activities are expressed in IUB units (U) per liter.

Fig. 1 The relation of sex and blood types to alkaline phosphatase levels in 247 males and 237 females at various ages.

Table 1 Serum Alkaline Phosphatase Activities and Sex Differences in Various Male and Female Age Groups

Age group	Males			Females			Sex
(years)	n	M	2 S. D.	n	M	2 S. D.	difference
10	92	82.8	44.6	98	93.3	48.2	Very Significant $P<0.001$
13	86	119.7	53.8	75	64.0	49.2	$P<0.001$
16	32	43.6	22.0	30	25.8	10.8	$P<0.001$
19	37	28.9	15.2	34	22.2	10.4	Not Significant $P>0.50$

n : number in group, M : mean, S. D. : standard deviation
The enzyme activities are expressed in U/liter.

gel was carried out on the basis of the method of Ornstein[8] in order to separate tissue-specific alkaline phosphatase isoenzymes. A physicochemical inhibition test for the isoenzymes was performed on the basis of the methods of Winkelman[9] and O'Carrol[10].

Results

The relation of blood type to serum alkaline phosphatase levels

Serum alkaline phosphatase activities in four age groups subdivided by sex and blood type are shown in Fig. 1. No statistically significant influence of blood type on enzyme activity was observed in both males and females of each of the four age groups. The following observation was obtained in males aged 13 years, for example, by t-test performed to confirm the validity of subdivision by blood type :

Between blood type A and B ($t=0.19$, $df=52$, $p>0.50$)

Between blood type B and O ($t=0.05$, $df=45$, $p>0.50$)

Between blood type O and AB ($t=0.69$, $df=30$, $0.50>p>0.40$)

Between blood type AB and A ($t=0.46$, $df=37$, $p>0.50$)

In females and other age groups, no significant blood type-related differences were also indicated as same as this observation.

Age and sex differences of values

Table 1 shows mean serum alkaline phosphatase activity and standard deviation for either sex and age group. In the same Table, the two sexes were compared by t-test with respect to mean enzyme activity for the total sample in each age group. At age 13 and 16, males generally had significantly higher mean serum alkaline phosphatase values than females. At age 10, contrarily, females had higher values than males. No significant sex difference was observed in the age of 19 years.

The mean enzyme activity was highest in females aged 10 years and in males aged 13 years. An abrupt decrease in enzyme activity was observed at age 19 in both sexes. The significant age-related differences between various age groups were obtained in both sexes. Comparison of various age groups by t-test is shown as follows :

Between age 10 and 13 ($t=4.18$, $df=182$, $p<0.001$ in male $t=8.82$, $df=165$, $p<0.001$ in female)
Between age 13 and 16 ($t=4.28$, $df=116$, $p<0.001$ in male $t=9.22$, $df=103$, $p<0.001$ in female)
Between age 16 and 19 ($t=7.30$, $df=64$, $p<0.001$ in male $t=3.11$, $df=65$, $0.001<p<0.01$ in female)

Estimation of isoenzyme by electrophoresis and physicochemical test

Physicochemical and electrophoretic studies of alkaline phosphatase isoenzyme were conducted with an unselected sera of boy saged 13 years and girls aged 10 years. The effects of heat, phenylalanine, EDTA, imidazole and urea inhibition on alkaline phosphatase activity of these sera are summarized in Table 2.

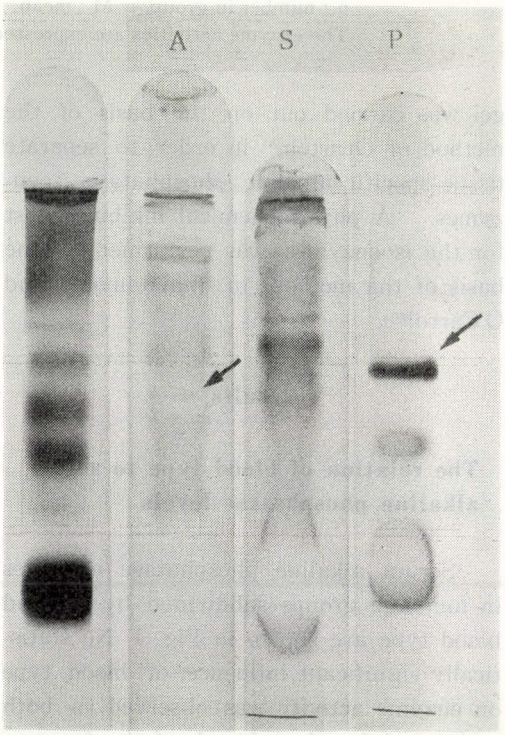
On serum alkaline phosphatase from these boys and girls, L-phenylalanine at 5 mM concentration had little effect. This alkaline phosphatase was almost completely destroyed by heating at 56°C for 5 min. and was inhibited 7.3—37.4 % by urea, EDTA and imidazole. From the results obtained, this serum alkaline phosphatase was considered to be due to osseous isoenzyme.

The polyacrylamide gel electrophoretic patterns of alkaline phosphatase activity for one of the sera used avobe are shown in the Photograph with the migration of the serum proteins. The predominant

Table 2 Differentiation of Serum Alkaline Phosphatase isoenzyme Activity in Adolescents by Physicochemical Inhibition Tests

Inhibitors	Total activity remaining after treatment (%)	
	Male	Female
1) L-phenylalanine (5 mM)	70.9	71.4
2) Urea (3 M)	31.4	32.0
3) EDTA (1 mM)	7.3	8.2
4) Imidazole (5 mM)	37.4	35.5
5) Heating (5 min, 56°C)	6.9	6.7

Parentheses exhibit final concentration or condition of each inhibitor



Gels are double stained for enzyme activity and protein, which are incubated for 60min. in 2-amino-2-methyl-1, 3-propan-diol buffer (pH 10.2) with 5-bromo-3-indolylphosphate, p-toluidine salt as substrate and subsequently in 3 g/dl Ponceau 3 R. Allows show the peak of alkaline phosphatase activity. A : serum from male aged 19 years, S : serum from male aged 13 years, P : placental phosphatase as a marker.

Photograph. DISC-electrophoretic separation of serum alkaline phosphatase on polyacrylamide gel.

alkaline phosphatase electrophoretic peak found in the serum was in the haptoglobin region, wherease the serum from a male aged 19 years showed a Transferrin peak. A placental alkaline phosphatase specimen as the marker showed an intermediate peak between the two peaks.

Discussion

Age-, sex- and blood type-related effects relative to serum alkaline phosphatase have been demostrated by several investigators by the manual method of Kind-King, and by various other techniques[1)-6)]. Eastman and Bixler, recently, reported the normal range of serum alkaline phosphatase activity as measured by a newly accepted kinetic method[6)]. We could confirm high levels of serum alkaline phosphatase in adolescence by the improved kinetic method. There was a significant fall of the levels at the age of 19 years, and no significant difference with sex was observed.

At the age of 13 and 16 years, however, males generally have higher levels than females, and at the age of 10 years, the levels in females were higher than those in males. Since alkaline phosphatase levels at the age of 19 years are essentially similar to those in adults (our normal adult range=male 19.0−31.4 U/liter, and female 16.0—28.1 U/liter), it is clearly important when using alkaline phosphatase as a diagnostic aid to take into account this high levels seen normally in adolescence. As it is not precise whether the alkaline phosphatase "flare" is seen in groups in one-yearly steps, a similar age- and sex-related study is intended to be performed on the same individuals presently investigated for a further 3 years.

Male and female populations in present investigation consisted of children from urban and semirural areas. However, Round indicated in his cross-sectional survey of 624 schoolchildren that statistical analysis of alkaline phosphatase data showed no significant differences within groups between children from these two areas[4)].

Concerning alkaline phosphatase isoenzyme, it has been demonstrated that the significant rise in serum alkaline phosphatase levels as compared with adults between the ages of 10 and 13 years paralleted the adolescent growth spurt, and that the rapid fall to normal adult levels seen after 16 years was due to a reduction in bone phosphatase levels[4)-6)]. In our investigation by physicochemical inhibition and electrophoretic separation, high serum alkaline phosphatase levels in boys aged 13 years and in girls aged 10 years were also considered to be due to bone alkaline phosphatase. As can be seen in the references[9)-11)], L-phenylalanine inhibits the intestinal and placental fration markedly. However, liver and bone alkaline phosphatase are equally resistant to L-phenylalanine, wherease they are imidazole-sensitive. Heating and urea have a selective inhibitory effect on bone fraction, compared with liver fraction.

Electrophoretic separation of tissue-specific serum alkaline phosphatase also exhibits the increase in osseous isoenzyme from a boy at the age of puberty according to the zymogram by Smith et al[12)].

From these observations, it is important for reduction of the incidence of diagnostic confusion not only to indicate the precise normal value at each age but also to separate tissue specific isoenzyme from high alkaline phosphatase seen in puberty. We compared the serum alkaline phosphatase levels and isoenzyme characteristics in children with obesty at the age of 10 years with those in normal children at the

same age. They were selected by body fat volume (more than 30%) and Rohrer index (more than 160) from large populations in the present blood examination project on normal adolescents. Mean value for 8 boys was 103.8 U/liter (range 65.0—159.8) and values for 3 girls were 56.0, 82.9 and 103.8 U/liter, respectively. Although high mean activity of alkalin phosphatase in adults with obesty has been reported[13)-14)], similar high mean activity should be seen in puberty, because statistically significant decrease in the activity was observed after treatment of these boys and girls with running training for 3 months. From the profile of the physicochemical inhibition for serum alkaline phosphatase activity from one of the boys, high levels of alkaline phosphatase activity in their sera are also considered to be due to osseous isoenzyme.

The present data show high serum alkaline phosphatase levels and significant age and sex differences in normal adolescent Japaneses, and also necessities to take account of alkaline phosphatase isoenzyme for diagnostic aid.

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References

1) L. C. Clark and E. Beck : *J. Pediat.*, **36**, 335 (1950)
2) M. J. S. Langman and E. Leuthold : *Nature*, **212**, 41 (1966)
3) Y. Ohba, M. Sasaki and N. Ida : *Pediat. Jap.*, **8**, 1062 (1967)
4) J. M. Round : *Brit. Med. J.*, **21**, 137 (1973)
5) J. Kattwinkel, L. M. Taussig, B. E. Statland and J. I. Verter : *J. Pediat.*, **82**, 234 (1973)
6) J. R. Eastman and D. Bixler : *Clin. Chem.*, **23**, 1769 (1977)
7) K. Tanishima, Y. Minamikawa, N. Yokogawa and M. Takeshita : *Clin. Chem.*, **23**, 1873 (1977)
8) L. Ornstein : *Ann. New York Acad. Sci.*, **121**, 321 (1964)
9) J. Winkelman, S. Nadler, J. Demetriou and V. J. Pileggi : *Am. J. Clin. Path.*, **57**, 625 (1972)
10) D. O'Carroll, B. E. Statland, B. W. Steele and M. D. Burke : *Am. J. Clin. Path.*, **63**, 564 (1975)
11) S. Ihno and H. Suzuki : *J. Med. Technol. Jap.*, **16**, 1504 (1972)
12) I. Smith, P. J. Lightstone and J. D. Perry : *Clin. Chim. Acta*, **19**, 499 (1968)
13) M. Yoshitsugu, T. Iwasa and T. Sassa : *Jap. J. Clin. Path.*, **25** (Suppl.), 39 (1977)
14) A. Ito, K. Kanazashi and S. Ikawa : *Report Res. Cent. Phys. Educat.*, **2**, 248 (1974)