Serum Alkaline Phosphatase: Normal Values by Sex and Age

John Russell Eastman and David Blixer

We report here the normal range of serum alkaline phosphatase activity as measured by the method proposed by Hausamen et al. [Clin. Chim. Acta 15, 241 (1967)] with a much larger sample size than used in previous investigations. In the statistical analysis the sample population is subdivided by sex and age, two variables which are known to influence enzyme activity. No statistically significant influence of blood type on enzyme activity was observed. The normal range of enzyme activity is reported in percentiles.

Serum alkaline phosphatase (EC 3.1.3.1) is a mixture of isoenzymes contributed primarily by bone, liver, and intestine (1). Because of these contributions, serum alkaline phosphatase determination has been used to help distinguish between normal and disease states of these organs.

This paper reports the normal range of serum alkaline phosphatase activity as measured by a newly accepted kinetic method. The range is based upon a much larger sample than used in previous investigations and the simultaneous treatment of variables known to influence the enzymatic activity: age, sex, and blood type.

Methods and Materials

Serum was obtained from clinically normal subjects whose families were involved in twin and linkage studies conducted by the Department of Medical Genetics. All families segregating a gene known to affect alkaline phosphatase activity were excluded, including those with any history of Van Buchem disease (2), tuberous sclerosis (3), or hypophosphatasia (4). Also excluded were pregnant women and individuals with hepatobiliary or skeletal disorders. Samples from the remaining 285 individuals (130 males, 155 females) were analyzed. The age of the subjects ranged from less than 1 up to 79 years.

Erythrocyte ABO antigen was typed (5) to determine if the reported 15% increase in activity of the intestinal isoenzyme in the serum of persons with blood types B and O was statistically significant (6, 7).

The manual method we used in measuring alkaline phosphatase activity was that of Hausamen et al. (8), adopted with slight modification by both the Scandinavian and German societies for clinical chemistry (9, 10). The variation within groups of duplicate analyses or the relative magnitude of the within-group variance component, expressed as a percentage of the among-group variance minus the error or within variance divided by sample size, was 1.28% (II). This figure is based on duplicate determinations in each of 76 individuals.

A Unicam SP1800 spectrophotometer with a spectral bandwidth of 1.6 nm was used to continuously monitor the formation of the enzymatic reaction product, p-nitrophenol. Change in absolute absorbance at 404 nm was recorded vs. time with a Unicam AR25 linear recorder; the value obtained was averaged over 2 min and converted into IUB units (micromoles of product formed per minute per liter) by the following formula:

\[(\Delta A/min \times \text{assay vol})/(\text{m.m.a.} \times \text{sample vol}) \times 100 = \text{U/liter.}\]

Millimolar absorptivity of substrate (m.m.a.) equals 18.6 in this buffer system. The reaction temperature of 30°C was chosen to conform with recommendations from the Committee on Standards of the International Federation of Clinical Chemistry (12).

Results

We did regression analysis, with age as the independent variable and serum alkaline phosphatase activity the dependent variable, on all 285 samples and found a negative correlation \((r = -.601, P < .01)\). When the data were grouped by age, an abrupt and large decrease in enzymatic activity was observable at age 18 in males and age 17 in females. Based upon this result and the significant regression of enzymatic activity on age, the sample was divided into two groups at these ages. Group I consisted of 100 young individuals (50 boys and 50 girls) and Group II consisted of 185 older individuals, 80 men (18 years and older) and 105 women (17 years and older). To confirm the validity of this subdivision, a t-test was performed and the means of the two groups were found to be significantly different \((t = 28.09, df = 285, P < .001)\). No significant regression of enzyme value on age was found for either group. These two findings indicate that the subdivision by age substantially reduced the regression on age and confirm the empirically observed decrease in enzyme activity between ages 17 and 18.

Next, the two sexes were compared by t-test with respect to serum alkaline phosphatase activity for the total sample, which indicated no significant sex-related difference. However, because the t was close to significance \((t = 1.763, df = 283, 1 > P > .05)\) each of the two age groups was tested separately. A significant difference between sexes in enzyme activity was found only in the adult (Group II) population \((t = 2.234, df = 183, P < .05)\), with males having a higher enzymatic activity then females. On this basis, Group II was further subdivided by sex.

Thus, our sample was divided into groups according to both sex and age (Table I). Group II is composed of men more than 18 years old and women more than 17 years old, and it is subdivided by sex, men designated as group IIA and women as group IIB. Group I included all individuals of both sexes with ages less than those specified in group II and is also subdivided by sex, boys comprising group IA and girls group IB. This subdivision by sex, though not mathematically jus-
Table 1. Normal Values by Sex and Age

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Group I</th>
<th>Group IIA</th>
<th>Group II</th>
<th>Group IIA</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>185</td>
<td>80</td>
</tr>
<tr>
<td>Male/female sex ratio</td>
<td>1/1</td>
<td>all male</td>
<td>all female</td>
<td>.7619</td>
<td>all male</td>
</tr>
<tr>
<td>Male age range, years</td>
<td>0–18</td>
<td>0–18</td>
<td>.19–60+</td>
<td>19–60+</td>
<td>.19–60+</td>
</tr>
<tr>
<td>Female age range, years</td>
<td>0–17</td>
<td>0–17</td>
<td>18–60+</td>
<td>18–60+</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>335.4282</td>
<td>343.9312</td>
<td>326.9212</td>
<td>104.8381</td>
<td>111.2277</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>111.3787</td>
<td>119.0325</td>
<td>103.6647</td>
<td>33.5273</td>
<td>33.4763</td>
</tr>
<tr>
<td>Coefficient of skewness</td>
<td>.2210</td>
<td>.3777</td>
<td>-.1008</td>
<td>.7328*</td>
<td>.5670*</td>
</tr>
<tr>
<td>Coefficient of kurtosis</td>
<td>2.5780</td>
<td>2.0506</td>
<td>2.9875</td>
<td>3.4538</td>
<td>2.8089</td>
</tr>
<tr>
<td>Range between 10th–90th percentiles</td>
<td>187.2–480.4</td>
<td>196.8–505.8</td>
<td>174.5–481.6</td>
<td>65.4–163.2</td>
<td>69.5–167.4</td>
</tr>
<tr>
<td>Range between 5th–95th percentiles</td>
<td>169.9–515.7</td>
<td>163.7–574.0</td>
<td>123.0–501.9</td>
<td>56.0–170.3</td>
<td>62.1–175.8</td>
</tr>
</tbody>
</table>

* Deviated significantly from a normal distribution.

tified (t = .7625, df = 98, .5 > P > .4), is done for the benefit of the reader.

A two-way analysis of variance utilizing blood type and enzyme value both for the total combined sample and each subgroup was not found to be significant.

The coefficients of skewness and kurtosis listed for each group in Table 1 indicate the nongaussian distributions of the enzyme activity values in groups II, IIA, and IIB. Because of this and the small sample size per group (less than 300), we used the percentile method of Herrera (13) to determine the normal range of enzyme activity in each group as recommended by Martin et al. (14); these normal percentile ranges are also shown in Table 1.

Discussion

Age- and sex-related effects relative to serum alkaline phosphatase have been demonstrated by several investigators, both by a modification of the present technique (15, 16) and by various other techniques (17–21). The improved assay methodology of Hausamen et al. we could confirm these effects. Furthermore, by considering these effects simultaneously and using a much larger sample size than was used in previous studies (15, 16) we obtained more nearly accurate values for normal serum alkaline phosphatase activity as measured by this technique.

Kattwinkle et al. (15) observed that only the isoenzyme that originates from bone is responsible for the increase in total serum alkaline phosphatase activity from infancy through puberty. The partitioning by age of groups I and II appears to emphasize the rapid rate of skeletal growth in group I, and a much larger sample of group I individuals may justify further subdividing it by age. Such a subdivision would be correlated with changes in the rate of skeletal growth that are known to occur during the pubertal growth spurt (22).

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References