Total, Tartrate-Resistant, and Tartrate-Inhibited Acid Phosphatases in Serum: Biological Variations and Reference Limits

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We studied several factors affecting biological variation in serum acid phosphatases in a population of 1195 apparently healthy subjects four years old or older. We assayed total acid phosphatase activities in the presence of a transphosphorylating agent and using alpha-naphthyl phosphate as substrate. The main factors modifying total and tartrate-resistant acid phosphatases activities in serum are similar to those observed for total and bone alkaline phosphatases activities: age, sex, and hormonal state (puberty or menopause). The tartrate-inhibited acid phosphatase activity is, however, independent of biological variations. Finally, we propose reference limits for total, tartrate-resistant, and tartrate-inhibited acid phosphatases in serum.

Additional Keyphrases: sex- and age-related effects · reference interval · bone metabolism · estrogens · phosphate · alkaline phosphatase

Serum acid phosphatase (ACP, EC 3.1.3.2) activity, especially the tartrate-inhibitable fraction or the immunologically determined prostatic isoenzyme, is generally used as a marker of prostatic cancer (1–5). The tartrate-resistant fraction is increased in various pathological circumstances (6–9), and has been proposed as a marker of bone metabolism (10, 11). The importance of knowledge of biological-variation factors in interpretation of clinical laboratory tests is now well recognized, and several studies have noted the influence of age on ACP activity (12–15). However, relatively few authors have studied the changes in ACP activity as a function of the physiological state. Thus our objectives here were to find out which biological-variation factors (excluding drugs) affect the activity of ACP, to quantify the effects of these factors, and to define reference limits for total, tartrate-resistant, and tartrate-inhibited ACP in serum.

Materials and Methods

Samples

Blood was sampled between 08.00 and 09.00 or between 12.00 and 13.00 hours (before the midday meal) from the antecubital vein of supine subjects, with use of a tourniquet for as short a time as possible. The blood was collected into polystyrene tubes (one containing lithium heparinate, one with no anticoagulant), via a plastic tube, and without use of a syringe. The heparinized tubes were immediately centrifuged for 10 min at 2700 × g and the plasma thus obtained was quickly separated from the cells and used for determinations of alkaline phosphatase activities and phosphate concentrations within the following 3 h. The tubes without anticoagulant were left standing at room temperature for the minimum time required for complete coagulation. They were then promptly centrifuged at 2700 × g for 10 min after the clot was freed from the sides of the tube with a glass rod, if necessary. The serum was rapidly separated from the cells and was analyzed for ACP activities within the following 2 h. Hemolyzed and (or) icteric specimens were eliminated, to avoid possible analytical interferences (16, 17).

Analytical Methods

Total alkaline phosphatase activities and phosphate concentrations in plasma were determined with a SMA II continuous-flow analyzer (Technicon, Homestead, 55330 France), at 37 °C.

Total ACP activities in serum were measured according to a modification of Hillmann’s method (18), in a Cobas Bio centrifugal analyzer (Hoffmann-La Roche, Basle, Switzerland), at 30 °C (19). Reagent kits were provided by Bio-Mérieux, Charbonnières-Le-Bains, France (cat. no. 61541). The final concentrations (per liter) of reagents in the reaction mixture were 150 mmol of pH 5.4 citrate buffer, 200 mmol of pentanediol as transphosphorylating agent, 10 mmol of alpha-naphthyl phosphate as substrate, 2.5 mmol of Fast Red TR salt, and 1 mL of a nonionic detergent. The volume fraction of sample was 1:11. The reaction mixture was initiated with serum. After a lag phase of 180 s, the rate of absorbance increase was monitored at 405 nm for 5 min (20-s reading intervals). For each series of measurements, a reagent blank (with water instead of sample in the reaction mixture) was measured and subtracted from the results.

Tartrate-resistant ACP activities were determined in the same way but with 75 mmol of sodium tartrate present per liter in the reaction mixture. Activities of the tartrate-inhibited enzyme were obtained by calculating the difference between total and tartrate-resistant activities.

For comparison purposes, we measured total and tartrate-resistant ACP activities in several sera with a kit from Boehringer Mannheim, Meylan, France (cat. no. 125008), in which 4-nitrophenyl phosphate is used as substrate. This fixed-time technique was performed at 37 °C, and the final absorbances were measured with a Shimadzu UV 160 spectrophotometer (Roucaire, Velizy-Villacoublay, France).

We also assayed prostatic ACP mass concentration in some sera by immunoenzyme assay, using kits from Merck, Darmstadt, F.R.G. (cat. no. 15688). After the prostatic ACP is bound to specific antibodies, the enzyme activity is determined, with p-nitrophenyl phosphate as substrate. The concentrations of prostatic ACP are read on a calibration curve and expressed in micrograms per liter.

Population

Our population sample included 1195 individuals (590 males and 605 females), ages four years or more, who came to the Center of Preventive Medicine in Vandoeuvre-lès-Nancy for a health examination between June and November 1983. Subjects with no evidence of disease were identified from the files of the state’s health insurance fund in Nancy. These subjects were invited in family groups, and
about a third of our population sample consisted of children four to 14 years old.

These subjects had fasted overnight or for at least 5 h and were taking no drugs (including oral contraceptives).

Statistics

The hypothesis that the distributions conform to a gaussian law was verified by a $\chi^2$ fit test for the various subgroups (adults and children) of the population sample. For the statistical analysis of the parametric data we used Student's $t$-test for unpaired data, Welch's $t$-test, and the Fisher-Snedecor $F$-test.

Results

Analytical Variations

Short-term and long-term analytical variations were monitored by use of lyophilized control serum and pooled specimens of human serum. Within-run repeatabilities were 0.5% to 0.8% (means from 17.7 to 4.1 U/L, respectively) for total ACP, 0.5% to 1.8% (means from 9.2 to 1.9 U/L, respectively) for tartrate-resistant enzyme, and 1.9% to 2.3% (means from 8.2 to 2.2 U/L) for tartrate-inhibited ACP. The coefficients of day-to-day variation were quite acceptable: 4.7%, 6.0%, and 7.2% (means 17.3, 8.7, and 8.6 U/L) for total, tartrate-resistant, and tartrate-inhibited ACP, respectively.

Biological Variations

Descriptive analysis of the population distributions. The frequency histograms of total, tartrate-resistant, and tartrate-inhibited ACP in serum are shown in Figure 1 for males and in Figure 2 for females. Each group of the population, males and females, was divided into two subgroups, one with children four to 19 years old, and one with adults of 20 years and more. The distributions for most of these subgroups of subjects are symmetrical and follow a
gaussian pattern (as assessed by $\chi^2$ test, $P > 0.05$), whether total, tartrate-resistant, or tartrate-inhibited ACP is considered. The distribution was non-gaussian only in women older than 19 years old for total ($P = 0.01$) and tartrate-resistant ACP ($0.005 < P < 0.01$), and in boys four to 19 years old for the tartrate-inhibited ACP ($0.005 < P < 0.01$). The values for children are clearly higher than for adults, both for total ACP and the tartrate-resistant fraction. For adults, the ACP activities ranged from 1 to 5.5 U/L for total ACP and from 0.1 to 4.0 U/L for the tartrate-resistant fraction; for children four to 19 years old, the values ranged from 1.5 to 8.3 U/L and from 0.4 to 6.7 U/L for the total and tartrate-resistant ACP, respectively. Under these conditions, it is clear that the frequency histograms for the whole population, including adults and children, show skewed distributions for total and tartrate-resistant ACP. The tartrate-inhibited ACP activities measured in children and adults are comparable, and range from 0.1 to 3 U/L.

Variations according to age and sex. The variances observed in the different compared subgroups were similar (not significant by F-test). Under these conditions, the degree of statistical significance given by Student's t-test and Welch's t-test was identical. Therefore, we only present statistical data obtained with the t-test.

During childhood and adolescence, total and tartrate-resistant ACP in serum change in parallel (Figure 3). For males, the value of the median of the total ACP activity decreases significantly from 5.4 U/L in children four to nine years old to 3.4 U/L in adults of 20 to 29 years (corresponding mean values: $5.3 \pm 0.8$ U/L to $3.4 \pm 0.9$ U/L, $P < 0.001$), and from 3.8 to 2.1 U/L (means: $3.9 \pm 0.6$ U/L to $2.1 \pm 0.6$ U/L, $P < 0.001$) for the tartrate-resistant fraction. The observed changes in females (for the same classes of age as for males) are similar, with a decrease from 5.4 to 2.8 U/L (means: $5.3 \pm 1.0$ U/L to $2.9 \pm 0.6$ U/L, $P < 0.001$) for the total enzyme activities, and from 3.8 to 1.8 U/L (means: $3.9 \pm 0.8$ U/L to $1.8 \pm 0.4$ U/L, $P < 0.001$) for the tartrate-resistant ACP. The tartrate-inhibited ACP shows no statistically significant variation with age.

Sexual maturation also affects serum ACP activity. Thus, the mean values for total and tartrate-resistant ACP are clearly lower for girls between 12 and 15 years old who have reached the menarche than in girls of the same age who have not. Activities in pre-menarche girls are greater by about 31% ($P < 0.001$) for the total ACP activities, and by about 52% ($P < 0.001$) for the tartrate-resistant enzyme. There is no statistically significant difference for the tartrate-inhibited ACP (Table 1).

During adulthood, no statistically significant age-related difference was observed in men for either total, tartrate-resistant, or tartrate-inhibited ACP activities.

For women, values for total and tartrate-resistant ACP do not change between 20 and 49 years. For women older than 50 years, total ACP activities increase significantly from 3.1 ± 0.7 U/L (women 40–49 years) to 3.4 ± 0.7 U/L ($P < 0.01$) and the tartrate-resistant fraction from 1.9 ± 0.4 U/L to 2.2 ± 0.6 U/L ($P < 0.001$). This increase seems to be related mainly to menopause, as Table 2 shows. ACP activity in serum of post-menopausal women significantly exceeds ($P < 0.01$) that in pre-menopausal women by about 20% and 26% for the total and tartrate-resistant ACP, respectively. The tartrate-inhibited ACP activity is not related to age or sex. Values for total and tartrate-resistant ACP are slightly but significantly ($0.01 < P < 0.001$) higher for males than for females.

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**Table 1. Influence of Menarche on Results for Total, Tartrate-Resistant, and Tartrate-Inhibited Acid Phosphatase**

<table>
<thead>
<tr>
<th>Acid phosphatase activity, U/L</th>
<th>Total</th>
<th>Tartrate-resistant</th>
<th>Tartrate-inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Pre-menarche     *</td>
<td>27</td>
<td>5.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Menstruating *</td>
<td>42</td>
<td>4.2</td>
<td>0.89</td>
</tr>
</tbody>
</table>

*Girls 12 to 15 years old. *Statistical significance (Student's t-test): $P < 0.001$. 

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**Fig. 3. Variation in the median and the dispersion of total (A), tartrate-inhibited (B), and tartrate-resistant (C) acid phosphatases as a function of age in males ( ) and females ( )**
Table 2. Influence of the Menopause on Results for Total, Tartrate-Resistant, and Tartrate-Inhibited Acid Phosphatase

<table>
<thead>
<tr>
<th>Acid phosphatase activity, U/L</th>
<th>Total</th>
<th>Tartrate-Resistant</th>
<th>Tartrate-Inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Menstrual*</td>
<td>37</td>
<td>3.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Menopause*</td>
<td>19</td>
<td>3.6a</td>
<td>0.53</td>
</tr>
</tbody>
</table>
| *Women 45 to 55 years old. a Statistical significance (Student's t test): P <0.01.

females 10 to 49 years old. There is no statistically significant sex-related difference after 50 years of age, because of the increase of ACP activity in women over 50 years old.

The degree of overweight, as calculated by Lorenz's formula (20) seems to not influence ACP activity (data not shown).

Effects of drugs and xenobiotics (including alcohol and tobacco) were not studied.

Reference Limits

Reference limits for 95% of our population sample were defined according to the recommendations of the Société Française de Biologie Clinique (21).

Subjects drinking more than 44 g of alcohol per day, smoking more than 15 cigarettes per day, or more than 20% overweight according to Lorenz's formula were excluded. Subjects were fasting and were taking no drug, including oral contraceptives. Reference limits by age and sex for total serum tartrate-resistant and tartrate-inhibited ACP at percentiles 2.5, 50, and 97.5 are summarized in Tables 3 to 5.

Correlations with Other Biological Factors

We established coefficients of correlation between total, tartrate-resistant, and tartrate-inhibited ACP and some biological factors known to be related to growth: alkaline phosphatase (EC 3.1.3.1) and phosphate. The alkaline phosphatase activities and the phosphate concentrations were measured in plasma derived from the same blood specimen as the serum used for ACP determinations (see Materials and Methods).

For children 4 to 20 years old, coefficients of correlation (P <0.001) were highly statistically significant between total ACP and alkaline phosphatase (r = 0.53 for boys, r = 0.59 for girls) and between the tartrate-resistant fraction and alkaline phosphatase (r = 0.56 for boys, r = 0.64 for girls). Results were similar for the coefficients of correlation between total ACP and phosphate (r = 0.46 for boys, 0.62 for girls) and between tartrate-resistant ACP and phosphate (r = 0.45 for boys, 0.61 for girls).

For adults older than 20 years, the coefficients of correlation between total ACP and alkaline phosphatase were lower for men (r = 0.15, P <0.01) than for women (r = 0.38, P <0.001). Between tartrate-resistant ACP and alkaline phosphatase, the coefficient of correlation was not significant in the case of men (r = 0.11), but highly significant in the case of women (r = 0.33, P <0.001). There was no significant correlation between phosphate and total and tartrate-resistant ACP in adults.

The tartrate-inhibited enzyme did not correlate significantly with alkaline phosphatases and phosphate in any group of the population.

Discussion

Several authors have noted the importance of the influence of age in the interpretation of results for total and tartrate-resistant ACP activities, particularly in children (12-15). In this paper, we have tried to identify the main biological variation factors affecting total, tartrate-resistant, and tartrate-inhibited ACP activities in serum. From these data, we have established reference limits for total ACP and the two fractions.

None of the biological factors of variation that we studied influenced values for tartrate-inhibited ACP—in particular, age and sex had no effect. This suggests that the tartrate-inhibited fraction, as measured in a presumably healthy population, could not reflect the concentration of circulating prostate-derived isoenzyme. We observed no linear correlation between the results of the measured tartrate-inhibited ACP activities and those obtained by immunoenzyme assay for the same serum specimens in a subpopulation sample of 111 healthy subjects (adults and children, both sexes). The means (and SD) were 1.1 (0.42) U/L (tartrate-inhibited

Table 3. Reference Limits for Total Acid Phosphatase Activity (U/L) in Serum: 2.5, 50, and 97.5 Percentiles

<table>
<thead>
<tr>
<th>Age, y</th>
<th>n</th>
<th>2.5</th>
<th>50</th>
<th>97.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-9</td>
<td>91</td>
<td>3.9</td>
<td>5.2</td>
<td>7.3</td>
</tr>
<tr>
<td>10-14</td>
<td>87</td>
<td>3.9</td>
<td>5.4</td>
<td>7.6</td>
</tr>
<tr>
<td>15-19</td>
<td>72</td>
<td>2.5</td>
<td>4.3</td>
<td>6.9</td>
</tr>
<tr>
<td>20-29</td>
<td>37</td>
<td>1.6</td>
<td>3.3</td>
<td>5.9</td>
</tr>
<tr>
<td>30-39</td>
<td>78</td>
<td>1.9</td>
<td>3.3</td>
<td>5.0</td>
</tr>
<tr>
<td>40-49</td>
<td>46</td>
<td>2.3</td>
<td>3.3</td>
<td>4.9</td>
</tr>
<tr>
<td>&gt;50</td>
<td>39</td>
<td>2.2</td>
<td>3.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

*Prepubertal girls, †postpubertal girls, ‡premenopausal women, §postmenopausal women.

Table 4. Reference Limits for Tartrate-Resistant Acid Phosphatase Activity (U/L) in Serum: 2.5, 50, and 97.5 Percentiles

<table>
<thead>
<tr>
<th>Age, y</th>
<th>n</th>
<th>2.5</th>
<th>50</th>
<th>97.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-9</td>
<td>91</td>
<td>2.8</td>
<td>3.8</td>
<td>5.2</td>
</tr>
<tr>
<td>10-14</td>
<td>87</td>
<td>2.8</td>
<td>4.1</td>
<td>5.8</td>
</tr>
<tr>
<td>15-19</td>
<td>72</td>
<td>1.4</td>
<td>3.1</td>
<td>5.4</td>
</tr>
<tr>
<td>20-29</td>
<td>37</td>
<td>0.8</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>30-39</td>
<td>78</td>
<td>0.9</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>40-49</td>
<td>48</td>
<td>1.2</td>
<td>2.1</td>
<td>2.8</td>
</tr>
<tr>
<td>&gt;50</td>
<td>39</td>
<td>1.2</td>
<td>2.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*Prepubertal girls, †postpubertal girls, ‡premenopausal women, §postmenopausal women.

Table 5. Reference Limits for Tartrate-Inhibited Acid Phosphatase Activity (U/L) in Serum: 2.5, 50, and 97.5 Percentiles

<table>
<thead>
<tr>
<th>Age, y</th>
<th>n</th>
<th>2.5</th>
<th>50</th>
<th>97.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-9</td>
<td>250</td>
<td>0.3</td>
<td>1.4</td>
<td>2.3</td>
</tr>
<tr>
<td>20-49</td>
<td>181</td>
<td>0.4</td>
<td>1.2</td>
<td>2.3</td>
</tr>
<tr>
<td>&gt;50</td>
<td>38</td>
<td>0.4</td>
<td>1.3</td>
<td>2.9</td>
</tr>
</tbody>
</table>

*Prepubertal girls, †postpubertal girls, ‡premenopausal women, §postmenopausal women.
ACP and 0.14 (0.40) μg/L (prostatic ACP), all values falling within the physiological range.

Our results for total and tartrate-resistant ACP activities in serum confirm the great influence of age, particularly in young people as shown by Chen et al. (13). Using the same substrate (alpha-naphthyl phosphate), these authors showed that total ACP activity can mostly be ascribed to the tartrate-resistant fraction, which represents about 90–92% of the total ACP activity in children up to 12 years, 80% in children 12–18 years old, and 67% in adults. We calculated from our own data that the tartrate-resistant fraction represents about 75% of the total ACP activity in children up to 14 years for girls and 19 years for boys, and 64% in adults. The relationships observed between total or tartrate-resistant ACP activities and age in children agree well with published data obtained by using alpha-naphthyl phosphate or 4-methylumbelliferyl phosphate as substrate (14). However, because alpha-naphthyl phosphate is the preferred substrate for the prostatic ACP isoenzyme (22), and because the effect of 1,5-pentanediol as transphosphorylating agent appears to be more marked on the tartrate-inhibited ACP fraction (23), we compared the influence of age, as observed in this study, with data obtained by using 4-nitrophenyl phosphate as substrate. For this comparison we used a subpopulation sample of 93 males and females, ranging in age from four to 19 years. With the Bio-Mérieux kit, we observed a significant decrease in total ACP activities between children four to nine years old and adults 20 to 29 years old, of about 49% (mean values from 4.7 to 2.4 U/L), and of about 46% for the tartrate-resistant ACP activities (from 3.5 to 1.9 U/L). With 4-nitrophenyl phosphate used as substrate we found the same subjects (same serum specimens) a decrease of about 41% in the mean values for total and tartrate-resistant ACP activities (from 17 to 10 U/L and from 15 to 9 U/L, respectively). In children the influence of age noticed by others is also found when total and tartrate-resistant ACP activities are assayed by use of alpha-naphthyl phosphate in the presence of a transphosphorylating agent.

The relationships between total or tartrate-resistant ACP and alkaline phosphatase or phosphate in children corroborate the fact that variations in serum tartrate-resistant and total ACP activities are linked to age and bone metabolism. But age is not the sole factor affecting these values; sexual maturity also influences them, as shown by our data for prepubertal and postpubertal girls. The sex hormones, particularly estrogens, could also explain the sex-related differences we observed, i.e., statistically significantly higher activities in males than in females between 10 and 49 years, and the increase in total and tartrate-resistant ACP activities in women after menopause. We observed similar physiological variations when studying serum total and bone-originated alkaline phosphatase activities (24).

Finally, the major biological factors influencing total and tartrate-resistant ACP activities are age, sex, sexual maturity, and hormonal status in women—i.e., puberty and menopause. The knowledge of such factors of biological variation led us to propose reference limits appropriate to homogeneous sub-groups of population.

During the past 10 years, there have been considerable efforts to develop immunological methods for measurement of the prostatic ACP isoenzyme in serum (25–31) and, more recently, of the tartrate-resistant fraction (32, 33). Simultaneously, the conventional methods for determination of the catalytic activity of the total ACP or of its tartrate-inhibited fraction were improved, including the use of more-specific substrates of the prostatic isoenzyme (34–36), development of continuous monitoring technologies (17, 37–39), and use of certain alcohols as transphosphorylating agents (23, 40, 41).

Using a kit from Bio-Mérieux containing 1,5-pentanediol (200 mmol/L), we obtained higher reference limits for adults than the values given by Seiler et al. (42) with similar reagents but without a transphosphorylating agent. These authors established their upper reference limits in men as 1.5 U/L for the tartrate-inhibited ACP; our values are 2.5 U/L in men and 2.0 in women. In addition, for total ACP, these authors obtained values of 3.0 U/L in women (95th and 97.5th percentiles) and of 4.2 and 5.2 U/L in men (95th and 97.5th percentiles, respectively). Our corresponding values are 4.5 and 5.0 U/L for women and men over 19 years old, respectively (97.5th percentile). The relatively high value of 5.2 U/L given by Seilers et al. may be due to the presence in their population sample of boys 16 to 18 years old. Indeed, for boys 15–19 years old in our population our upper (97.5th percentile) value is 6.9 U/L.

In conclusion, we have defined the main sources of biological variation of the serum ACP activities and we stress the importance of having reference limits appropriate to individuals, particularly for the serum total ACP and its tartrate-resistant fraction.

We thank Bio-Mérieux, Charbonnières-Les-Bains, France, for the supply of kits (Phosphatase acide cinétique cat. no. 61541) for this study. We gratefully acknowledge Mrs. M. P. Recouvré and C. Raval for their technical assistance and Mrs. M. J. Longis for statistical analyses.

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