BIOLOGICAL VARIATIONS OF XANTHINE OXIDASE AND MYELOPEROXIDASE IN STERILE HUMAN URINE

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ABSTRACT

The analytical, intra-individual and inter-individual variations were determined for xanthine oxidase (XO) and myeloperoxidase (MPO) and the reference values were established. A total of 380 apparently healthy people, 177 male and 203 female (from 20 to 82 years, average 40 years old), were randomly selected from villages and cities of the southern part of Turkey. XO and MPO activities in human sterile urine samples were measured by spectrophotometric method. The distributions were Gaussian and no significant difference was observed between the male and female subjects. The mean value (standard deviation) of the population investigated for XO was 414.0 (93.3) U/L, and for MPO 104.5 (49.2) U/L, respectively. The analytical, intra-individual and inter-individual variations were assessed in 20 apparently healthy subjects and were found to be; XO: 6.2 %, 21.2%, and 19.2% MPO: 4.0%, 16.4%, 11.7% respectively. The results of the index individuality showed that reference values of XO and MPO could be used for diagnostic purposes in various diseases.

Key words: Sterile urine, xanthine oxidase, myeloperoxidase, biological variations

INTRODUCTION

Oxygen free radicals could be generated by a variety of reasons at the cellular levels. An important source of oxygen free radical is known to be xanthine oxidase (XO) that could be formed from xanthine dehydrogenase either reversibly (via oxidation or blockage of its thiol groups) or irreversibly (via limited proteolysis) under pathological conditions. The aforementioned conversion reaction leading to the elevated tissue XO levels is thought to be responsible for mechanism of several pathological conditions including alcoholism and smoking. Polymorphonuclear (PMN) leukocytes themselves are an important source of reactive oxygen species causing cellular injury. It was reported that
free radicals leading to pathological lesions in the kidneys could be generated via infiltration of neutrophils. Detection of PMN leukocytes infiltration in an inflamed tissue has been based on the measurement of the myeloperoxidase (MPO) activity. Biological variation have been used for many purposes in clinical chemistry-including setting of analytical goals, deciding the significance of changes in serial results, and assessing the utility of conventional population-based reference values. There are many data on the biological variation of analytes in serum or plasma, but few regarding analytes in urine. To the knowledge of the ours, none of the previous researches investigated the biological variations of XO and MPO levels in human urine. Therefore, in this study was done to determining the reference values for urine XO and MPO enzymes analytical variation (CV_A), intraindividual variation (CV_I), and inter-individual variation (CV_G). Also this paper provides data on within-subject and between-subject biological variation of XO and MPO for determining the index of individuality and critical differences on which to decide whether the quantity of the analytical has an used for diagnostic purpose.

MATERIALS AND METHODS

Subjects

The subjects were randomly selected from villages and cities of the east mediterranean region of Turkey. All were apparently healthy and were taking no drugs. Because this study was designed to investigate the components of biological variation in normal ambulant individuals, no restrictions were imposed on fluid intake, diet, or physical activity. The reference population (380) as healthy individuals was consisted of 177 male and 203 female (from 20 to 82 years, average 40 ages). Reference individuals chosen for the survey were in a state of good health defined by the International Federation of Clinical Chemistry (IFCC). Kahramanmaras Sutcu Imam University Medical Faculty Ethical Committee approval was taken in accordance with the principles of Declaration of Helsinki and informed consent was obtained from the cases.

Urine samples

The spot urine samples of subjects were collected into 75-mL sterile containers (Kayline Plastics, The barton, South Australia, 5031). The samples were transported to the laboratory within 30 minutes and were divided into two aliquots for microbiologic and biochemical analyses.

Analytical techniques

A. Microbiologic analysis

Urine samples were collected in the morning by midstream catch or by a catheter with strict aseptic technique. Gram-stained smears of unspun urine were examined in order to assess the presence of bacteria and leucocytes, as a guide to the presence of infection. The urines were cultured quantitatively on MacConkey’s agar and blood agar plates. Arbitrarily, concentrations of $10^5$ and more bacteria per 1 ml of urine were considered as
significant for urinary tract infection. Bacteria isolated in cultures were subjected to further identification procedures.

**B. Biochemical analysis**

XO activity was determined spectrophotometrically according to the method of Prajda and Weber \(^{11}\), based on the formation of uric acid from xanthine, which increases absorbance at 292 nm (\(\varepsilon M = 9.2 \pm 10^3\)). One unite of activity was defined as 1\(\mu\)mol of uric acid formed per minute and data are presented as U/L. Determination of MPO activity was carried out spectrophotometrically using 4-aminoantipyrine/phenol that is a substrate for MPO-mediated oxidation by H\(_2\)O\(_2\). Absorbances were read at 510 nm, and the data were given as U/L \(^{12}\).

Calculation of results

Using analysis of variance techniques, we divided the total variance into the components attributable to analytical variance, intra-individual variance, and interindividual variance. We used Student’s unpaired t-test to assess whether the means for men and women were different. Bonferroni test as Post Hoc was used for determining the difference according to age in reference population. Linear regression analysis was used to look for significant trends in values for the levels of XO and MPO and to investigate the time dependence of the intra-individual variations.

**C. Analytical variation**

10 ml of urine sample taken in sterile cabses was divided into two portions. An aliquot was taken each day for triplicate analysis of XO and MPO which was performed for 10 consecutive days. The calculated variance gave the inter-assay variation. The second portion was divided into 15 aliquots and each aliquot was studied on the same day. The calculated variance gave the intra-assay variation. The total analytical variation was the sum of intra- and inter-assay variances.

**D. Intra-individual and inter-individual variation**

Twenty healthy laboratory staff took part in the study voluntarily. Seven urine samples were taken from each individual on the 1st, 3rd, 5th, 7th, 15th, 30th days. The intra-individual (CV\(_I\)) and the inter-individual variation (CV\(_G\)) were calculated by nested analysis of variance (ANOVA).

**RESULTS**

There was no urinary tract infection in any of the urine samples. Normal distributions of reference population were done for XO and MPO using Kolmogrow Smirnov analysis. All distributions were found as normal. There were no significant differences in mean activities of XO and MPO with respect to age or sex. The means (S.D.) and ranges of urine XO and MPO enzymes for reference population investigated were shown in Table 1.
Table 1. The activities of urine xanthine oxidase and myeloperoxidase in the reference population.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>mean</th>
<th>SD</th>
<th>median</th>
<th>range</th>
<th>min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine oxidase (U/L)</td>
<td>414.0</td>
<td>93.3</td>
<td>403.5</td>
<td>589</td>
<td>156-745</td>
</tr>
<tr>
<td>Myeloperoxidase (U/L)</td>
<td>104.5</td>
<td>49.2</td>
<td>103.0</td>
<td>254</td>
<td>20-82</td>
</tr>
</tbody>
</table>

The analytical variation (CV_A%), analytical goal (≤1/2CV_I%), index of individuality (CV_I/CV_G) and critical difference (2.77 (CV_A^2+CV_I^2)^1/2 %) were shown in Table 2. The intra-individual variations of XO and MPO, we found for men and women over 30 days are no different in subjects. We believe that this constancy of intra-individual variation means that (a) existing data on biological variation can be validly used for a number of purposes, and (b) setting up complex experiments with large numbers of subjects is not necessary for deriving valid data on overall intra-individual variation.

Table 2. Calculated components of variation and indices derived from data on biological variations of xanthine oxidase and myeloperoxidase in reference population

<table>
<thead>
<tr>
<th></th>
<th>Xanthine Oxidase (U/L)</th>
<th>Myeloperoxidase (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analytical variations</td>
<td>6.2</td>
<td>4.0</td>
</tr>
<tr>
<td>Intra-individual variations</td>
<td>21.2</td>
<td>16.4</td>
</tr>
<tr>
<td>Inter-individual variations</td>
<td>19.2</td>
<td>11.7</td>
</tr>
<tr>
<td>Analytical goal</td>
<td>10.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Index of individuality</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Critical difference</td>
<td>61.1</td>
<td>46.7</td>
</tr>
</tbody>
</table>

* The analytical variation (CV_A%), analytical goal (1/2CV_I%), index of individuality (CV_I/CV_G) and critical difference (2.77 (CV_A^2+CV_I^2)^1/2 %).

**DISCUSSION**

XO enzyme is confined in man mainly to the liver; a significant but lower activity has been found also in jejunal mucosa and colostrum; on the other hand, the kidney, prostate, and blood elements, as well as normal serum, are virtually devoid of XO activity. Indeed, Giler S et. al. showed that urinary XO activity related specifically to pathogenic urinary bacteria, increased activity being present only in the urines containing bacteria in amounts greater than 10^5 per ml. In our that XO activity was found to be negligible in sterile human urines (from 156 to 745 units, as presently defined, per litre) (Table 1). Giler S et al. reported that XO activity in urinary tract infection increased 2-3 fold. MPO enzyme is derived from the primary azurophil granules of neutrophils.
MPO though is capable of directly reacting with superoxide and neutralising free radicals generated during the inflammatory response \(^\text{16}\). In our study, MPO level in sterile urines found to be 104 unite per litre. Fraser PA et al., \(^\text{17}\) showed that a population of symptomatic and asymptomatic men had a wide range of urinary MPO levels without any discriminative value. Also, they reported that MPO levels within urethral secretions might therefore be "selfregulating" and not show disease association. Although there are numerous published papers about levels of XO and MPO in various diseases, yet little is known about the factors that might influence XO and MPO levels under physiological conditions. Therefore, it is necessary to define biological fluctuations within an individual and the reference ranges of the population for XO and MPO levels in urine as previously done for other analysates such as amylase, isoamylase, total lipid, cortisol and prothrombin \(^\text{18} - \text{19}\).

Many studies of biological variation in serum analytes \(^\text{20} - \text{21}\) have shown that such variation is independent of age, race, lifestyle, and analytical procedures. Fraser and Harris \(^\text{22}\) described the applications of biological variability estimations in the clinical field. Measurements of urine constituents and of their biological variability \(^\text{23} - \text{25}\) have been considered less relevant than serum constituents in the clinical laboratory. Some work have been done in establishing quality specifications for urine testing \(^\text{26}\) and in deriving the practical applications of biological variation data \(^\text{27}\).

Several investigators have proposed \(^\text{28} - \text{30}\) that analytical goals should be derived from biological variation data. For analyses used in testing or monitoring an individual, it has been proposed that the allowable analytical variance should be less than or equal to one-half of the average intra-individual variation \(^\text{31} - \text{32}\). Analytical goals so derived from our
results are shown in Table 2. To our knowledge, this is the first definition of analytical goals for urinary XO and MPO analytes based on biological variation data. The goals of XO and MPO derived in this study from biological variation experiments may be ubiquitously applicable for beginning of a database on this biological fluid.

Although commonly requested clinical chemistry tests are likely to be of little value in diagnosis of minor illness, in practice most test requests to laboratories are made for the purpose of monitoring patients. In that context, it is vital to know the magnitude of change in serial results from an individual that makes a difference statistically significant. Such changes are due to analytical and within-subject variation; for \( P \leq 0.05 \), the critical difference is \( 2.77 \left( \frac{CV^2_A}{CV^2_I} \right)^{1/2} \% \). The critical differences are also listed in Table 2. The question arises as to whether clinicians appreciate the magnitude of these critical differences. The results of a recent survey of the opinions of clinicians allow calculation of the median percentage of critical differences considered to be clinically important. In our study, although the critical differences documented in Table 1 may be used as a simple single figure to guide clinical decision making, they are not ubiquitously valid. No heterogeneity in XO and MPO in our study. The critical differences of XO and MPO are valid and all subjects have the same within-subject variation.

The dispersion of conventional reference intervals is due to a composite of analytical, intra-individual, and inter-individual variation. When the index of individuality \( (CV_I/CV_G) \) is less than 0.6, reference values are of limited utility, but, when \( CV_I/CV_G \) is more than 1.4, such population-based reference values are of considerable use (20, 21, 22). For a high index of individuality, >1.4, it has been said that reference intervals will be more useful than for a low index, < 0.6. The accepted criterion for diagnosis is the comparison of a single test value with the population-based reference interval. As Table 2 shows, for XO and MPO, the index of individuality is >1.4, the discriminant value proposed by Fraser and Harris (22); hence XO and MPO analytes can be useful for diagnosis.

**CONCLUSION**

From the data obtained in this study we conclude that:

* There were no significant differences in activities of XO and MPO with respect to age or gender.
* Analytical goals derived from data on biological variation for XO and MPO may be ubiquitously applicable in initiating a database on this biological fluid.
* The calculated indices of individuality for XO and MPO were the index of individuality is >1.4, the discriminant value proposed by Fraser and Harris (22); hence XO and MPO analytes can be useful for diagnosis.
* No heterogeneity in XO and MPO was found. The critical differences of XO and MPO were valid and all subjects had the same within-subject variation.

**COMPETING INTERESTS**

The author declares no competing interest.
REFERENCES