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Research Article

Hyperuricemia revisited in Split, Croatia

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Abstract

Purpose: The current upper reference interval (RI) limits for serum uric acid in Croatia seemingly do not reflect the actual situation, are too low, and may instigate overdiagnosis and overtreatment with potential global implications.

Methods: The study was conducted prospectively (during 2014) in 13 family medicine practices in Split, Croatia. The examinees' sera were processed using standard uricase technology. The sample consisted of 4,834 laboratory results; 2,469 pertained to apparently healthy and 2,365 to other patients. The data were tabulated, stratified according to age and gender, and statistically analyzed.

Results: The observed uric acid levels were consistently above the upper, official RI limits for Croatia (337 μmol/l for women and 403 μmol/l for men); over 15% of the results were definitely hyperuricemic, being higher among men and increasing in older age groups, particularly in women.

Conclusions: The actual RIs for uricemia are indeed too low for the investigated south Croatian population and should be increased by some 50 μmol/l or more in most age/gender strata. Even the proposed, upgraded RIs may be of assistance in clinical judgment only, and should never be used as the exclusive criterion for clinical decisions.

Abbreviations: RI- reference interval; AH- apparently healthy; CDL- clinical decision limit

Introduction

Serum levels of uric acid are determined by dietary intake of purines, their endogenous metabolism, urinary excretion of urates and intestinal uricolysis, which depend on various hereditary factors, general health condition, sex, age, body mass and surface area, ethnic and geographic peculiarities, as well as individual life style, nutritional habits, and medication [1-3]. Hyperuricemia positively correlates not only with gout but with arterial hypertension, and with other conventional cardiovascular risk factors, such as obesity, diabetes mellitus type II, dyslipidemia and metabolic syndrome [4,5]. However, it is often interconnected with other enhancing elements (e.g. insulin resistance, lower glomerular filtration, many diuretics used in the management of arterial hypertension), so that causal relationship is not clear [1,2].

Medical decisions are often founded on laboratory tests, and it is crucial to distinguish "normal" from "abnormal" results. The distinction is usually based on the reference intervals (RIs, i.e. ranges of a laboratory test values in a particular "healthy", reference population), obtained from more or less representative samples (e.g. students, nurses, blood donors and other volunteers). The spread of such results often differs from the symmetric distribution, does not represent all the population's strata, and seldom is absolutely reliable [6]. Most of the RIs include the 95th percentile of "healthy" persons from the reference population and exclude the remaining 5% [6]. Overlapping between "healthy" and "sick" is inevitable, and lowering the upper limits increases the test sensitivity but decreases its specificity, and *vice versa*.

Comprehensive serum uric acid RIs for the various regions of southeastern Europe have not been established [7-9]. Textbook RI statements are discordant in this respect as well [10]. For example, eight reference books quote the upper RI limits for men at 361, 393, 403, 420, 476, 480, 506 and 536 μmol/l (μmol/l ÷ 59.48=mg/dl) [9]. The official upper RI borders in Croatia are currently ≥ 337 μmol/l for women, and ≥ 403 μmol/l for men [11]. These limits originate from data obtained many years ago in the northern part of Croatia (Zagreb region) [7,8]. Regional differences in uric acid concentration may be quite large, as recently shown in China [12].

Over the last few years the authors of this report were consulting a growing number of asymptomatic patients labeled hyperuricemic according to the national RIs. Presuming that this overdiagnosis trend was due to inadequate upper RI limits, we have planned to assess the actual distribution of uricemia in family medicine patients scheduled for routine laboratory tests. Our hypothesis was that the levels of serum uric acid among adults in Split region are significantly above the current Croatian reference values [11], possibly around the preset limits of ≥380 μmol/l for women and ≥430 μmol/l for men, extrapolated from the literature data [1-3,9,10].

Materials and Methods

This observational study was done in 2014 in 13 family medicine practices in Split, which use the service of two accredited laboratories [13]. Both laboratories used enzyme colored quantitative method on Olympus analyzer (laboratory 1: Beckman Coulter AU 680, Brea,

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California, USA; laboratory 2: Beckman Coulter AU 680, Mishima, Japan). The photometric quantification of uric acid concentration (PAP) was based on uricase, converting uric acid to alantoin and hydrogen peroxide, which produces a chromophore, read bichromatically at 660/800 nm [14].

The sample consisted of all consecutive, adult persons sent for laboratory testing from January 1, 2014 to December 31, 2014, except those with a malignant disease or suffering from renal insufficiency. In individual test-lists, in addition to uric acid value, age and gender, data on diagnosis of gout and drugs taken for four months preceding the laboratory test date were recorded. Apparently healthy (AH) examinees had to have no diagnosis, condition, or medication in their documentation that could have any impact on uricemia and were mostly persons attending check-up visits or pre-employment medical examination.

The research was approved by the Split-Dalmatian County Health Center's Ethics Committee. This board decided that individual informed consent was not necessary for this investigation since the laboratory tests were not performed for the study's own sake, common practice does not require patient consent for standard lab testing, and the data were anonymous, related to the healthcare code numbers only.

The uric acid level results were stratified in seven ages and two sex categories. The results were tabulated in Microsoft Office Excel and processed statistically with SPSS 17.0.1 (SPSS Inc., Chicago, IL, USA). Since the distribution of uricemia and age subgroups significantly deviated from normal according to Kolmogorov-Smirnov test (P=0.003), the statistical analysis used nonparametric measures of central tendency (percentiles, median and interquartile range) and appropriate, two-sided significance tests (Kruskal Wallis, Mann-Whitney U, χ^2); P <0.05 was considered statistically significant.

Results

Included in this research were single test results from 4,834 examinees (1,310 results were excluded from the initial 6,144 because of multiplicity or incompleteness): 2,713 (56.1%) women and 2,121 (43.9%) men, between 18 and 96 years of age with a median of 61 years. Majority of them were AH individuals: 2,469 or 51.1%. This subgroup was significantly younger than the rest (Mann-Whitney U between subgroups, P < 0.001).

AH women (Table 1), particularly in the older age subgroups have urate levels markedly above the national RIs (i.e. 337 μmol/l), surpassing even our preset upper limits (i.e. 380 μmol/l). Their slope becomes steep in the 5th decade.

Among AH men (Table 2), substantially elevated serum uric acid concentrations, i.e. above 403 or 430 mol/l, are even more prevalent, especially in younger persons, ranging from 5% to 19%, depending on age (χ^2= 59.546; P <0.001). The age-related increase observed in women was not registered in men. In fact, a slight decline in the upper percentiles of urate distribution (χ^2= 1.370; P= 0.240) was noted in elderly males.

Table 3 displays the distribution of uricemia in the subgroups according to relevant health status. The subset of AH examinees had a lower median relative to all other strata (with drugs and diagnoses that affect uricemia) [3], but even in them the 95th percentile exceeds the current upper reference limits in both genders. The observed differences between groups, although statistically significant, were less than expected.

Discussion

The higher the plasma levels of uric acid the greater the likelihood of acute gouty arthritis, urate nephropathy and untoward cardiovascular outcome. However, the prognostic relevance of plasma uric acid concentration is controversial. On one side a group of researchers claims that treatment of asymptomatic hyperuricemia is beneficial because it may lower the health risk [4,15-18], while on the other side some scholars label it ill-advised because of potential harm from the currently available interventions and loss of the potential antioxidant effect [5,19-22] It is not clear at what level of uricemia is the preventive drug intervention advisable. Contemporary guidelines do not recommend such treatment in asymptomatic subjects unless uricemia exceeds certain critical values (≥ 594 μmol/l for female and ≥ 773 μmol/l for male patients) [10, 23].

Table 1. Uricemia distribution by age in apparently healthy women (N=1,473).

Age (years)	N	Median	Percentiles			Share above RIs*	
			75th	90th	95th	A	B
18 - 29	138	248.0	277.0	301.1	328.3	4%	1%
30 - 39	235	237.0	270,0	311.0	336.2	4%	1%
40 - 49	285	229.0	269.5	298.4	325.7	3%	1%
50 - 59	329	253.0	295.0	330.0	353.5	8%	3%
60 - 69	299	272.0	316.0	368.0	387.0	14%	6%
70 - 79	129	280.0	334.0	368.0	397.0	24%	5%
80 - 96	58	283.0	353.0	381.4	397.0	25%	10%
Total	1,473	253.0	293.0	336.0	368.0	9%	3%

*RIs= upper limits of the reference interval; A= uricemia ≥ 337 μmol/l; B= uricemia ≥ 380 μmol/l.

Table 2. Uricemia distribution by age in apparently healthy men (N=996).

Age (years)	N	Median	Percentiles			Share above RIs*	
			75th	90th	95th	A	B
18 - 29	107	338.0	391.0	427.2	483.8	18%	8%
30 - 39	144	341.0	394.8	443.5	495.8	19%	12%
40 - 49	205	336.0	377.5	437.4	477.9	16%	11%
50 - 59	198	341.5	386.0	434.5	488.5	18%	10%
60 - 69	202	341.0	384.0	419.0	435.9	15%	8%
70 - 79	100	340.5	384.8	417.9	437.8	18%	9%
80 - 96	40	324.5	354.5	407.6	434.1	10%	5%
Total	996	339.5	384.0	427.3	457.5	17%	10%

*RIs = upper limits of the reference interval: A = uricemia ≥ 403 μmol/l; B = uricemia ≥ 430 μmol/l.

Table 3. The effect of drugs on uricemia (N= 4,834).

Category*	A	B	C	D	E	F	AH
N	730	374	565	47	170	479	2,469
5th percentile	196.3	182.0	216.3	270.2	283.6	249.0	180.0
10th percentile	226.0	205.0	240.0	291.3	313.0	288.0	200.0
25th percentile	274.0	248.0	284.0	333.5	355.0	352.0	237.0
Median	334.5	300.0	338.0	383.5	418.0	424.0	286.0
75th percentile	392.0	352.0	395.0	439.3	484.0	489.0	342.3
90th percentile	450.5	407.5	452.5	509.7	561.6	554.0	393.0
95th percentile	489.8	438.9	484.8	536.0	622.0	585.0	427.0
IQR	118.0	104.0	111.0	105.8	129.0	137.0	105.3
P: A - F vs. AH#	<0.001	0.186	<0.001	<0.001	<0.001	<0.001	

*Category A= subgroup with drugs that increase uricemia, B= subgroup with drugs that decrease uricemia, C=subgroup with drugs that increase and decrease uricemia, D=subgroup taking allopurinol without gout, E=gouty subgroup taking allopurinol, F=gouty subgroup without allopurinol, AH=apparently healthy. #P values with Kruskal-Wallis ANOVA test.

The purpose of this study was to find out whether the existing reference values (RIs) should be revised, and to underscore the crucial difference between the statistically defined RIs and clinical decision limits (CDLs). The actual reference values, which are sometimes uncritically accepted as suggestions for therapeutic intervention, may lead to overdiagnosis and overtreatment of individuals with asymptomatic, mildly elevated serum uric acid levels. Indeed, in the tested population from the Split region the existing benchmark values seem to be inappropriate (biologically and statistically too low). The overall prevalence of such, descriptively defined hyperuricemia [7-11] is well over 15% in Split and more prevalent among men. We have observed a steady increase in uric acid levels with age, particularly steep in apparently healthy women after menopause, which was recently demonstrated by another study in southern Croatia [24].

Concomitant therapy did not influence urate levels as much as expected. Moreover, gouty patients taking allopurinol (almost the only hypouricemic drug available at that time) were having higher uricemia than their counterparts not taking the drug (Table 3). These results may be due to poor adherence (non-compliance) with the prescribed medication or inadequate diagnosis of gout for any suspicious arthropathy accompanied by "high" serum urates, without proper polarized microscopy of the joint exudates [9,10,25].

As previously mentioned, serum uric acid RIs vary widely by region, ethnicity and other variables. Our impression is that overdiagnosis and hasty overtreatment of asymptomatic "hyperuricemia" may be due to two factors: a) extrapolation from other conditions (e.g. the current guidelines on primary prevention in hyperlipidemia or arterial hypertension), which suggest therapeutic intervention well below the population upper RI limits because of proven therapeutic gain (i.e. improved prognosis), and b) wrong assumption that laboratory results above the upper RI limit (often too low, derived from "ideally healthy" individuals) are tantamount to CDLs, i.e. sufficient to warrant clinical intervention for prevention of gout flare-up, acute kidney failure or cardiovascular complications.

Even if a positive correlation between high levels of uric acid and atherosclerotic complications were undeniably determined, the link would not mean causation. Moreover, it is not clear whether reduction of elevated urate levels with the currently available tools may improve prognosis or perhaps have a negative impact, causing serious side effects, significant cost and other problems by exposing legions of our fellow citizens to such treatment. Well-designed clinical trials are definitely needed, especially with the recent, presumably better tolerated drugs. [2,25].

Finally, Table 4 suggests some interim, upper uric acid distribution limits resulting from this investigation [26].

Table 4. Tentative upper RI limits for serum uric acid (μmol/l) in Split, Croatia

Age (years)	Women	Men
18-29	320	385
30-39	325	405
40-49	330	420
50-59	375	435
60-69	390	450
70-79	415	470
80+	450	490

These margins largely surpass the official RIs, even our preset limits (380 and 430 μmol/l) and reflect age and gender peculiarities. In order to correspond to the actual plasma urate distribution in Split region, the official limits in many age/gender strata should be raised a lot. Similar results have been obtained in other countries; for example, the upper 95% percentile RI limit in north-eastern India is estimated at 428 μmol/l for women and 488 μmol/l for men [27]. Canadian upper limits for the 13 - 79 age range have been determined at 369 μmol/l for women and at 458 μmol/l for men [28]. The already mentioned Chinese study [12] has shown a remarkable regional variability in plasma uric acid upper limits: from 394 to 474 μmol/l for the female, and from 495 to 599 μmol/l for the male gender. Of course, the confines proposed on Table 4 request further verification with proper sampling [6,29]. Even these revised margins may be currently too low for any CDL recommending a medical intervention, except life-style modifications (general measures, dietary restrictions) [10,25].

This study has several limitations. First of alls it is observational. Second, the recommended procedures for validation of RIs [29] have not been followed. Instead, consecutive, single data obtained from a large outpatient pool were analyzed, which in strict statistical terms was a convenience sample that may not formally represent and/or be directly extrapolated to the target population. In fact, our aim was not to determine new, robust uricemia RIs but to check whether the actual ones are really too low. Proper selection of a "representative" sample is another conundrum. For instance, due to declining health status with advancing age, 79% of the potential Canadian examinees aged 60 to 79 years were eliminated from the Canadian Health Measures Survey reference intervals study according to exclusion criteria [28] In other words, almost 80% of the elderly were deemed "ill", "sick" or simply "abnormal" for the sake of setting strict RIs [29]. Are so obtained, "ideal" data really representative of the target population? On the other hand, the advantage of our investigation is the large amount of verifiable data which may reliably reflect the Split population as they are derived from every individual tested for uric acid plasma levels, as a part of routine laboratory work-up.

Conclusions

1) There are substantial discrepancies among the existing RIs for serum uric acid levels in human plasma; some of them are due to biologic variability, some are presumably outdated, and most were obtained from samples of strictly defined healthy individuals.

2) The Croatian uricemia RIs [11], based upon results obtained more than two decades ago in northern part of the country [7,8] are definitely too low for the southern Croatian population and should be notably raised in some age/gender strata. Regional differences, age and sex subsets must be considered [29].

3) Even the modified, upgraded serum uric acid RIs are no more than a statistical description and a helpful tool in clinical judgment, which may never become the decisive parameter for crossing the Rubicon between health and disease, between observation and intervention.

4) The problem of overdiagnosis is becoming global, and remarkable regional differences in serum uric acid concentration shown in this and other reports [9,12,27,28] may stimulate productive discussion, scholarly criticism, and further clinical investigation.

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Declaration of interest

The authors report no conflicts of interest (including financial and other relationship). We alone are responsible for the content and writing of the paper. Relevant ethical issues were solved before the inception of this study.

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