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Assessment of reference values for selected elements in a healthy urban population

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Summary. - Reference values for 26 elements, namely Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn and Zr are proposed in serum and blood of 110 healthy adults of the urban area of Rome. They were included in the study on the basis of strict criteria of eligibility and exclusion. With the exception of Ba, Bi, Co, Cr, Ni, Sb, Sn Tl in serum, and Bi, Hg, Si, V and W in whole blood, experimental data for each all the other analytes were found to approach a normal distribution. The estimated 5 - 95% references ranges (in ng ml^{-1}) were reported. For several elements the reference ranges observed overlapped information available in the literature. Gender, age, body mass index, smoking habits and alcohol consume were used as grouping variables. Mutual associations were observed for several elements, as follows: Be, Ca, Co, Cr, Cu, Li, Mo, Pb and Zn with sex; Ca, Pb and Si with age (< and > 45 years); Co, Cr, Mo, Sb and Tl with body mass index; Cd and Pb with smoking habit; Cr and Pb with alcohol consume.

Key words: trace elements, reference values, serum, blood, healthy subjects.

Riassunto *(Stima dei valori di riferimento di elementi chimici in una popolazione urbana sana)*. - Sono riportati gli intervalli di riferimento per Al, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, Hg, Li, Mg, Mn, Mo, Ni, Pb, Sb, Si, Sn, Sr, Tl, V, W, Zn e Zr in siero e sangue. Centodieci adulti sani abitanti nell'area urbana di Roma sono stati selezionati sulla base di rigidi criteri di idoneità ed esclusione. I dati sperimentali per tutti gli analiti, con l'esclusione di Ba, Bi, Co, Cr, Ni, Sb, Sn e Tl (siero), e Bi, Hg, Si, V e W (sangue), presentano una distribuzione normale. I valori di riferimento sono riportati come 5 - 95% percentili. Per molti elementi gli intervalli di riferimento osservati coincidono con quelli reperibili in letteratura. La popolazione è stata anche suddivisa secondo le variabili sesso, età, indice di massa corporea, fumo e consumo di alcol. Molti elementi evidenziavano le seguenti mutue associazioni: Be, Ca, Co, Cr, Cu, Li, Mo, Pb e Zn con il sesso; Ca, Pb e Si con l'età (< e > di 45 anni); Co, Cr, Mo, Sb e Tl con l'indice di massa corporea; Cd e Pb col fumo; Cr e Pb col consumo di alcool.

Parole chiave: elementi in traccia, valori di riferimento, siero, sangue, popolazione sana.

Introduction

Reference values (RVs) for major and trace elements in human body fluids make possible a better understanding of data deriving from environmental and occupational monitoring as well as clinical practice [1]. In fact, it is expected that they are an expression of natural levels affected by age, sex, habits, living and working environments, and diseases. The knowledge of RVs of chemical elements in human turns out to be key factor for an effective control of exposures and an improved knowledge of the potential role of metals in health disorders. Numerous concentration intervals claimed to be typical for the healthy population groups, but sometime they differ by up to an order of magnitude for certain elements. This can be due to problems associated with the characterization of reference group, as well as with the sampling, pre-analytical and quantification procedures used.

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By definition, RVs are related to a well-defined group of individuals [2-5]. Inadequacy and inhomogeneity of the reference group composition often makes the acquired data of scant scientific quality, scarce for regulatory resolutions and useless for studying trace element-related diseases. Reference groups should include subjects, which can be volunteers, students, blood donors, etc., not occupationally exposed to the elements under evaluation. It is not possible to say *a priori* which group is the most suitable as reference group in a particular investigation; ideally it should be randomly recruited among individuals similar to the group under study in respect to age, gender, general exposure, and many other variables but not in relation with disease or kind of exposure to be evaluated. Therefore, the first prerequisite is a detailed description of the subjects, carried out by means of exhaustive questionnaire.

Moreover, the robustness of the resulting RVs seriously depends not only on the final instrumental determination, but also on pre-analytical procedures adopted, i.e., on the collection, storage, handling and treatment of the samples. Main risk associated with these pre-analytical steps is the modification of the analytical information, in terms of contamination of the samples or loss of the analytes. The pre-treatment procedures, therefore, should combine ease of operations with low contamination risk, e.g., wherever possible the use of adequate disposable vessels in which carry out the whole manipulation (sample collection, digestion/dilution and determination) should be preferred. Assessment of RVs involves the examination of a large number of specimens, thus, to apply routine analytical procedures with high throughput is also crucial. Moreover, the combination of multi-element quantification capabilities and good detection power of the inductively coupled plasma spectrometry techniques with atomic emission or mass detection (ICP-AES or ICP-MS, respectively) makes these systems favoured for epidemiological screenings. In light of the above considerations, this paper reports the ascertainment of the reference ranges for 26 elements in serum and blood of volunteers living in the urban area of Rome.

This investigation could contribute to assess, for people living in a urban area, the entity of exposure to toxic metals and baseline of the essential elements.

Experimental

Subjects selection

Questionnaire fit for the choice of the population was applied in order to achieve homogeneous healthy group. Informed written consent was obtained from each subject and relating personal data were entered in an anonymous format. The aspects of the life (environmental exposures, lifestyles, dietary habits, therapies, medical history, etc.) which could affect the element burden in the organism have been considered (see Table 1). The adopted exclusion criteria were the following: cardiological, respiratory, kidney or liver disorders; intestinal absorption abnormalities; active infections; assumption of thyroid hormones or lithium; psychoactive drug intake; assumption of vitamins or mineral integrators; iatrogenic exposure from metallic implants such prostheses, surgical screws or intra-uterine inserts. Subjects resulting out of the physiological ranges about chemical-clinical parameters were also excluded.

Table 1. - Main information required for recruiting reference healthy subjects

Identification
ID code
Sex - Age - Height - Weight - BMI
Current domicile (how long)
Current job (how long, place)
Anamnesis
Severe or chronic pathologies in the past (age of diagnosis)
Late drug assumption (within 60 days)
Dental filling (amalgam, resin, alloys, etc.) (how long, number)
Metal prosthodontics (type, how long, number)
Other metal prostheses (type, how long, number)
Habits and lifestyle
Trinkets use
Aluminum pots use
Exercise (type, frequency)
Traffic intensity at the domicile
Distance from industrial areas
Smoke (type, quantity, frequency)
Alcohol assumption (type, quantity, frequency)
Diet
Type (predominant food)
Fish consumption (predominant food)
Milk and dairy products consumption (predominant food)
Within 2 years body-weight variations (>10 kg)
Parents-pertinent information
Father/mother age at birth-date
Smoke (type, quantity, frequency)
Health conditions
Alive or death age

Collection, processing and analysis of the haematological samples

In general, the entire experimental scheme was designed taking strict precautions to avoid alteration in the analytical determination of the elements. Briefly, the safety measures included the use of the following set of devices and reagents: *i)* latex gloves powder-free; *ii)* teflon endovenous catheters; *iii)* polystyrene disposable tubes, previously decontaminated; *iii)* hydrogen peroxide and nitric acid of suprapur grade; and *iv)* deionised water. Blood drawings were executed within 8 and 10 a.m. on subjects fasted overnight, and the venipuncture area was disinfected by means of hydrogen-peroxide and then rinsed with deionized water. The first portion of blood (about 100 or 300 ml) was kept for blood donation and other parameters determination, the remaining aliquot (10 ml) was used for the elements quantification. This procedure allowed to rinse catheter and pipes and further minimize contamination of elements which can be transferred to the blood during collection. Blood and resulting serum samples were soon frozen at -20 °C and preserved until the processing and analysis. All treatment procedures were performed in a Class 100 clean laboratory. Among all the element quantified, Al, Ba, Be, Bi, Cd, Co, Cr, Hg, Li, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Tl, V, W and Zr were determined by sector field ICP-MS whilst those at major concentration such as Ca, Cu, Fe, Mg, Si and Zn were analysed by ICP-AES. A deeper description of pre-analytical and analytical topics - and relating quality control and performances - has been reported in a previous paper [6].

Statistical treatment

Simple descriptive statistics as mean, standard deviation (SD), 5% trimmed mean, median and percentiles were applied to the analytical data obtained. The normality of the results were tested by using the Kolmorogov-Smirnov statistics (K-S test). Age (below and above 45 yrs), sex, alcohol consume and smoking habits were treated as grouping variables in the ANOVA or Mann Withney *U*-test. Moreover, age and body mass index (BMI) were correlated with elements burden by Pearson or by Spearman procedures as a function of the type of data distribution. The statistical package SPSS 12.0 (SPSS Inc., Chicago, IL, USA) was used for the analysis.

Results and discussion

Study population

The main characteristics of the study population are resumed in Fig. 1. Among the 110 selected blood donors, aged between 20 and 61 yrs (mean 42 ± 10 yrs), 37 were women (mean age 39 ± 10 yrs and mean BMI 23) and 73 men (mean age 44 ± 9 yrs and mean BMI 26); 30 (8 women and 22 men) were smokers or ex-smokers from less than 5 yrs; and 49 (13 women and 36 men) drunk one or more glasses *die* $^{-1}$ of wine or equivalent amount of alcohol.

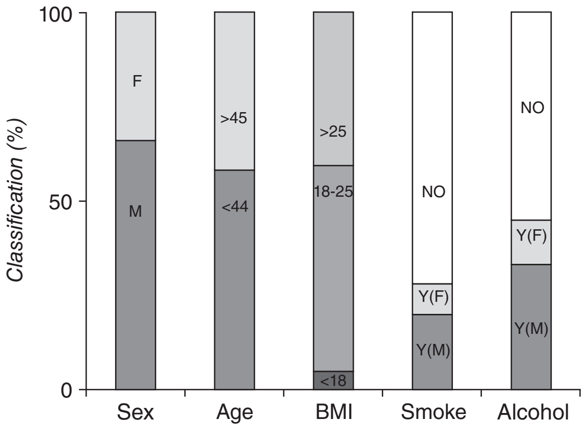

Fig. 1. - Characteristics of the population under study. F: females; M: males; Y: yes; BMI: body mass index.

Assessment of the reference ranges

In general, trace element composition of the serum and whole blood provided good approximation to the normal distribution according to the K-S test, although in some cases the skewness toward higher values and the deviation of the kurtosis became important. In particular, statistical analyses were carried out by using parametric tests for all analytes, whilst in the cases of Ba, Bi, Co, Cr, Ni, Sb, Sn and Tl in serum, and Bi, Hg, Si, V and W in whole blood the non-parametric statistics were applied, because of their non-normal distribution.

Tables 2 and 3 give the basic statistical treatment of data for serum and blood, respectively. The 5% trimmed mean, i.e., the mean of the data in the 5 - 95% percentiles, is also reported as it is scarcely affected by the influence of the skewness in both directions. The comparison between means (normal or 5% trimmed) and medians for each analyte further corroborates the evidence of a good central tendency for almost all analytes, with the exception of Ba, Ni and Sn in serum, and Hg and Si in blood. As general rule, reference ranges are based on the percentiles concept or on the intervals obtained from 5% trimmed mean ± 2 SD. Here, the percentiles approach was preferred. As expected, the reference ranges calculated from 5 - 95% fractiles resulted to be nearly equivalent to those obtainable with 5% trimmed mean, this providing further evidence of the nearly symmetric distribution

Table 2. - Concentration ranges (ng ml^{-1}) of trace elements in serum

Element	Mean ± SD	5% trimmed mean	Median	Fractiles (%)				Literature data *(*)*
				5	25	75	95	
Al	2.33 ± 1.51	2.24	2.03	0.43	1.17	3.09	5.29	0.5 - 8
Ba	0.67 ± 0.33	0.65	0.56	0.32	0.43	0.87	1.37	0.4 - 1.7
Be	0.21 ± 0.12	0.21	0.20	0.06	0.13	0.31	0.43	0.03 - 0.27
Bi	0.02 ± 0.01	0.02	0.02	0.01	0.01	0.02	0.03	*(**)*
Ca	63,055 ± 5,858	63,248	63,755	52,577	59,035	67,702	70,632	91,000 - 106,000
Cd	0.10 ± 0.05	0.09	0.09	0.03	0.06	0.12	0.20	0.04 - 0.36
Co	0.19 ± 0.11	0.18	0.16	0.06	0.10	0.25	0.42	0.03 - 0.41
Cr	0.16 ± 0.07	0.16	0.15	0.07	0.11	0.19	0.28	0.04 - 0.48
Cu	947 ± 238	932	933	648	776	1,072	1,301	601 - 1,803
Fe	1,688 ± 493	1,701	1,651	886	1,396	2,087	2,455	825 - 2,090
Hg	1.32 ± 0.72	1.29	1.28	0.32	0.80	1.70	2.75	0.6 - 3.8
Li	1.09 ± 0.63	1.04	0.97	0.36	0.62	1.54	2.20	<0.1 - 7.25
Mg	17,603 ± 1,779	17,651	17,368	14,643	16,485	19,030	20,255	17,000 - 22,000
Mn	0.62 ± 0.23	0.61	0.60	0.31	0.46	0.71	1.02	0.3 - 1.48
Mo	0.86 ± 0.46	0.84	0.81	0.19	0.50	1.15	1.69	0.27 - 0.91
Ni	0.46 ± 0.36	0.43	0.36	0.10	0.17	0.63	1.25	0.13 - 2.8
Pb	0.54 ± 0.25	0.53	0.53	0.20	0.34	0.69	0.98	0.1 - 0.5
Sb	0.10 ± 0.07	0.10	0.09	0.02	0.06	0.14	0.22	0.01 - 1.7
Si	132 ± 63	129	129	46.9	82.2	160	245	*(**)*
Sn	0.69 ± 0.43	0.65	0.53	0.27	0.35	0.94	1.69	0.3 - 2.6
Sr	39.4 ± 13.1	38.8	36.8	23.0	28.7	46.2	61.5	28 - 44
Tl	0.04 ± 0.02	0.04	0.04	0.02	0.03	0.06	0.09	0.02 - 0.34
V	0.06 ± 0.03	0.06	0.06	0.03	0.04	0.08	0.11	0.02 - 0.11
W	0.03 ± 0.02	0.03	0.03	0.01	0.02	0.05	0.06	<0.004 - 0.02
Zn	812 ± 131	811	820	597	717	899	1,028	587 - 1,215
Zr	0.11 ± 0.05	0.11	0.12	0.04	0.08	0.14	0.19	0.03 - 0.22

(*): ranges including data from references [7-12]; *(**)*: not available.

for the majority of the variables under study. The RVs obtained could be roughly divided into two groups, respectively, the first group for which ranges substantially coincided with the literature data, and the group rather deviated from intervals reported by other authors, as included in the Tables 2 and 3 [7-12]. More specifically, Ba, Be, Hg, Li, Mg, Mo, Sr, V, W and Zn showed good agreement with the published results for both serum and blood, whilst serum Co, Cu, Mn and Zr as well as blood Ca, Cd, Cr, Fe, Mn, Ni and Zr practically overlapped the respective intervals existing in literature.

In this survey Al, Cd, Cr, Ni, Sn and Tl in serum, and Co, Cu, Li and Sn in blood showed reference intervals narrower than those available in literature with a tendency to shift to low limit of the ranges. Moreover, trace elements such as Ca, Sb and Tl in serum, and Bi, Sb and Tl in blood displayed concentrations basically out of the lowest limit of the corresponding published ranges. These facts could be explained as a consequence of the strict criteria adopted for subjects selection as well as of the attention paid to the contamination problems along the entire analytical sequence.

Conversely, a few elements showed reference ranges faintly larger than the corresponding interval found in the literature, and tended to exceed its upper limit. This is the case of Fe and Pb in serum, and of Al, Cr and Pb in whole blood. No firm explanation can be offered for the higher Fe levels here found in serum, except for that relating to the characteristics of the population under study. This discrepancy - which is slightly lessened

Table 3. - Concentration ranges (ng ml-1) of trace elements in blood

Element	Mean ± SD	5% trimmed mean	Median	Fractiles (%)				Literature data *(*)*
				5	25	75	95	
Al	17.0 ± 9.37	16.5	15.3	5.93	9.68	21.2	33.3	2 - 15
Ba	1.25 ± 0.61	1.22	1.13	0.50	0.80	1.66	2.40	0.47 - 2.4
Be	0.42 ± 0.19	0.42	0.44	0.10	0.28	0.51	0.75	<0.07 - 0.28
Bi	0.03 ± 0.02	0.03	0.03	0.02	0.02	0.04	0.06	0.12 - 0.80
Ca	66,332 ± 10,817	66,103	65,150	52,197	59,028	72,193	87,258	55,900 ± 6,400
Cd	0.99 ± 0.51	0.96	0.95	0.25	0.59	1.32	1.97	0.1 - 1.7
Co	0.12 ± 0.08	0.11	0.11	0.03	0.06	0.15	0.24	0.04 - 0.91
Cr	0.44 ± 0.27	0.43	0.38	0.12	0.26	0.55	1.07	0.01 - 1.2
Cu	938 ± 141	940	935	686	854	1,025	1,157	780 - 1,760
Fe	549,839 ± 60,937	549,815	547,589	453,519	510,018	583,334	646,491	390,000 - 550,000
Hg	6.36 ± 4.11	6.09	4.94	1.97	3.33	8.75	14.5	1.7 - 9.9
Li	0.86 ± 0.54	0.83	0.71	0.20	0.46	1.19	1.87	1.2 - 3.4
Mg	40,420 ± 5,309	40,386	40,329	32,824	36,951	43,276	49,276	32,700 ± 1,500
Mn	7.70 ± 3.13	7.71	7.85	1.53	5.93	9.50	13.2	7.1 - 10.5
Mo	3.06 ± 1.57	3.00	2.88	1.02	1.83	4.11	6.03	0.21 - 5.41
Ni	0.89 ± 0.61	0.86	0.83	0.14	0.43	1.21	2.13	0.3 - 3.3
Pb	39.5 ± 20.2	38.7	35.0	12.8	25.3	50.5	79.5	4 - 47
Sb	0.47 ± 0.26	0.46	0.41	0.07	0.29	0.66	0.94	0.3 - 3.0
Si	160 ± 68	156	142	85.4	112	197	277	*(**)*
Sn	1.51 ± 0.60	1.49	1.48	0.63	1.10	1.82	2.61	0.35 - 4.3
Sr	27.3 ± 11.8	26.9	25.5	10.1	19.6	34.9	50.3	7 - 36
Tl	0.07 ± 0.04	0.07	0.07	0.03	0.05	0.10	0.15	0.15 - 0.63
V	0.09 ± 0.05	0.08	0.08	0.03	0.05	0.11	0.18	0.03 - 0.28
W	0.07 ± 0.03	0.07	0.06	0.03	0.04	0.09	0.14	0.02 - 0.18
Zn	6,717 ± 924	6,713	6,597	5,189	6,134	7,365	8,337	4,076 - 7,594
Zr	0.49 ± 0.24	0.48	0.45	0.20	0.32	0.62	0.95	0.07 - 0.44

(*): ranges including data from references [7-11]; *(**)*: not available.

considering 25 - 75% intervals - might reflect the analogous Fe higher concentration observed in blood of the same subjects. However, the range here established corresponded to 5 - 95 percentiles found by Forrer *et al*., i.e., 825 - 2090 ng ml^{-1} [12]. Similarly, Al, Cr and Pb contents here found in blood can be reasonably considered inside the published ranges if the restricted fractiles (25 - 75%) were taken into account. However, all these differences could be plausibly explained by the features of the populations tested as well as by the weak divergence found.

Reference values vs *grouping variables*

The analysis of the effects of sex, age, BMI, smoking habit and alcohol consume on the elemental concentrations were also performed. Marked sex-related differences (p = 0.01) between males and females for Cr, Cu and Zn in serum, and for Be, Ca, Co, Cu, Li, Mo and Pb in whole blood were observed. In particular, means ± SD of these metals, grouped by sex, tended to be significantly higher in males for serum Cr and blood Mo and Pb, whilst for all the others elements were lower in males (see Table 4). Only for some analytes previous results in function of the gender are available. Serum Cu and blood Co and Cu levels were higher in females than in males, as well as serum Zn and blood Pb concentration was lower in females, as showed also by other authors [12-16]. In addition, blood Ca higher burden found in females could be associated with activity of female sex hormones, as suggested by Gafter *et al.* [17]

Table 4. - Significance of the difference in the elements concentrations (ng ml^{-1}) as a function of the grouping variables ($p < 0.005$)

Matrix/Element	Grouping variable	
	Gender	
	Males	**Females**
Blood/Be	0.38 ± 0.18	0.50 ± 0.19
Blood/Ca	62,958 ± 9,231	73,172 ± 10,672
Blood/Co	0.10 ± 0.06	0.16 ± 0.09
Blood/Cu	913 ± 126	996 ± 159
Blood/Li	0.74 ± 0.50	1.10 ± 0.55
Blood/Mo	3.36 ± 1.51	2.44 ± 1.50
Blood/Pb	46.8 ± 19.7	25.3 ± 11.4
Serum/Cr	0.17 ± 0.07	0.14 ± 0.06
Serum/Cu	886 ± 186	1,076 ± 284
Serum/Zn	841 ± 126	755 ± 123
	Age	
	< 45 yrs	**> 45 yrs**
Blood/Ca	68,801 ± 10,871	62,820 ± 9,824
Blood/Pb	30.9 ± 15.6	51.3 ± 19.9
Serum/Si	146 ± 65	112 ± 55
	Smoke	
	Yes	**No**
Blood/Cd *(*)*	1.16 ± 0.54	0.93 ± 0.49
Blood/Pb	46.6 ± 19.7	36.7 ± 19.8
	Alcohol	
	Yes	**No**
Blood/Cr *(*)*	0.51 ± 0.30	0.38 ± 0.23
Blood/Pb	46.8 ± 21.4	33.9 ± 17.3

(*) : statistical significance of the difference, $p < 0.02$.

When age was considered as grouping variable, subjects elder than 45 yrs showed significantly lowered levels of Si (serum) and Ca (blood) and increased concentrations of Pb (blood), as shown in Table 4. This pattern was fully confirmed by the correlation analysis. In fact, serum Si and blood Ca levels were inversely associated to the age of the subjects (at $p = 0.02$ and $p = 0.001$, respectively), while blood Pb concentrations were positively correlated ($p = 0.001$). Due the lack of available data for Si, no comparison with other investigations was possible. Since most of the Ca is located in plasma/serum (about 85%), it was reasonable to stress that only a rather small difference of this metal in the organism occurred with the age. This was supported by the weak statistical significance observed in serum and by the published data, which are thought to be contradictory and inconclusive about the relation Ca/age [18]. The behaviour of blood Pb levels with age, i.e., their increase along the lifetime, was in good agreement with data of other authors [19].

Subjects categorized by the smoking habits showed, as expected, significant differences in the Pb and Cd blood contents, with higher elements burden for the smokers. Regular smokers had, in fact, mean blood Cd and Pb level ca. 20% more that of people who never smoked, as shown in Table 4. Similar evidences are reported in the literature, although blood Cd in smokers was reported to be 2 or 3-times higher than in non-smokers [20, 21].

The alcohol consume was determinant for Pb and Cr blood content, but its impact on the latter's level was more moderate. This confirms the well known effect of alcohol intake on blood Pb and its important role as source of exposure [19, 22].

Lead levels both in serum and in blood were strongly correlated with the BMI. Moreover, Pb showed, as a function of the BMI increase, the tendency to accumulate into the cellular portion of the blood more than in the serum. This correlation has not been found by Weyermann *et al.* [23]. Analogously, robust associations with the increasing BMI were showed, in serum, by Cr (positive) and Sb (inverse), and in blood by Mo and Tl (positive) and Co (inverse). No other correlations with elements level in haematological compartment and BMI were here found.

Conclusions

These data provide systematic information about the elemental status of a urban population. Due to the fact that the subjects included in the study were a large group of selected adults, these findings can be considered as reference ranges of the corresponding population. Moreover, it should be noted that reference intervals here observed overlapped the published RVs, with few minor exceptions. Finally, when the element status was evaluated as function of potential determinants as age, sex and etc., the correlations found suggested interesting associations which, however, in some cases should be corroborated by further experimental evidence.

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