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Correlation between blood and hair lead levels in boys and girls of Sardinia (Italy)

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Summary - *The aim of this study was to analyse the correlation between blood lead (PbB) and hair lead (PbH) in Sardinian children. The sample consisted of 330 children (126 boys and 204 girls) from three Sardinian towns with different environmental backgrounds: Portoscuso, Sant'Antioco, and Sestu. The boys of Portoscuso have the highest median value of PbB (10.86 µg/dL), followed by the girls of Portoscuso (7.24 µg/dL); they are followed, but with much lower values, by the boys of Sant'Antioco (4.22 µg/dL) and Sestu (4.06 µg/dL), and lastly by the girls of Sant'Antioco (3.50 µg/dL) and Sestu (3.39 µg/dL). There is a similar pattern for the PbH values: the Portoscuso boys have the highest median value (10.00 µg/g), followed by the Portoscuso girls (7.21 µg/g), Sant'Antioco boys (5.44 µg/g), Sant'Antioco girls (4.69 µg/g) and finally the Sestu boys (3.79 µg/g) and girls (1.56 µg/g). The values of the Bravais-Pearson coefficient of correlation between logPbB and logPbH are statistically significant both for the total sample ($r=0.4351$; $p\leq0.001$) and for the sexes considered separately ($r=0.3989$, $p\leq0.001$, for males; $r=0.3801$, $p\leq0.001$, for females). It should be noted that a high percentage of unexplained variance persists in the total sample (81.07%) and in males (84.09%) and females (85.55%) separately. The pattern among samples with different environmental backgrounds and the significant correlations between the logPbB and logPbH values suggest that hair can be used as a suitable biomarker of lead exposure.*

Keywords - *Lead, Blood, Hair, Boys, Girls, Sardinia.*

Introduction

Hair has been widely used as a biological material for the analysis of metal contents in an organism. Therefore, it is considered to provide information about the degree of exposure to environmental contaminants (Chatt & Katz, 1988; Bergomi *et al.*, 1989; Senofonte *et al.*, 1989; Vienna *et al.*, 1995; Foo & Tan, 1998; Lekouch *et al.*, 1999; Furman & Laleli, 2000; Nowak & Chmielnicka, 2000; Strumylaite *et al.*, 2004).

Nevertheless, there is extensive debate about the limitations of hair as a biomarker of metal exposure (Renshaw, 1976; Ryabukin, 1978; Morton *et al.*, 2002; Barbosa *et al.*, 2005). In fact, the Pb concentration profile has been found to vary significantly among various subpopulations according to age, sex, hair colour and smoking status (Wolfsperger *et al.*, 1994). Moreover, geographical, ethnic and ecological factors can also affect the Pb distribution in hair within a given population. Thus, it is difficult to establish reference ranges because confounding factors impose restrictions on the interpretation of individual results. For example, there is no consensus on the length of the hair specimen

to be collected, or the amount, or the position on the scalp. Variations in Pb content between single hairs from the same individual can be as high as ± 100%, particularly in the distal region (Renshaw, 1976; Barbosa *et al.*, 2005).

Furthermore, it is still debated whether there is good concordance between the levels of metals measured in hair and in biological fluids (Wilhelm & Idel, 1996; Lech, 2002; Sanna *et al.*, 2003; Barbosa *et al.*, 2005).

However, the technique of heavy metals determination in hair has given rise in the last decade to studies in various fields, such as toxicology (Taylor, 1986), medicine (Klevay *et al.*, 1987; Wecker *et al.*, 1985), clinical dietetics (Dorea *et al.*, 1982; Kozielec *et al.*, 1989) and environmental pollution (Wilhelm *et al.*, 1988; Bergomi *et al.*, 1989; Schumacher *et al.*, 1991; Golow & Kwaansa-Ansah, 1994; Revich, 1994; Srivastava & Gupta, 1994; Chlopicka *et al.*, 1995; Olejnik *et al.*, 1997; Nowak, 1998; Sanna *et al.*, 2003; Hasan *et al.*, 2004).

The aim of this study was to evaluate the correlation between lead levels in hair (PbH) and blood (PbB) using data for boys and girls from three Sardinian towns with different environmental backgrounds.

Subjects and methods

Sampling

The investigation was conducted in April and May 1998. The total sample consisted of 330 subjects (126 males and 204 females), 117 from Portoscuso (53 males and 64 females), 108 from Sant'Antioco (47 males and 61 females) and 105 from Sestu (32 males and 73 females). All the children (from 11 to 14 years old) had been resident in their respective municipalities for at least 5 years.

The subjects were children attending the middle schools of the respective towns whose parents consented to the data collection and who did not present characteristics that can alter the blood lead concentration (e.g. smokers, drinkers of alcoholic beverages, etc.). The three subsamples represented 52% of the 11-14-year-old children of Portoscuso, 24% of those of Sant'Antioco and 14% of those of Sestu in 1998.

The calendar age of each subject was converted to decimal age according to Eveleth & Tanner (1990).

Blood lead

Blood samples were obtained by venipuncture; each specimen was heparinized immediately and stored at 6° C. At the appropriate time, the material was analysed on a Perkin-Elmer absorption spectrophotometer, Model 1200 (AAS), equipped with a chart recorder, graphite furnace HGA 400, autosampler A40, and deuterium arc lamp background corrector. Qualitative control of the blood analyses was performed with BCR-194 and BCR-195 standards (certified values of 126 and 416 µg/L, respectively, in bovine reconstituted blood, European Union Community Bureau of References).

Hair lead

Hair samples (total sample weight per individual of about 1 g) were taken from the occipital region (nape). The hair was cut near the scalp and the first 2-5 cm of recent growth were used. Each sample was preserved in a carefully sealed plastic bag, labelled with a progressive number, date of birth, sex and place of residence of the individual. All specimens were stored in a cool, dry, ventilated environment until delivered to the laboratory, where they were kept in desiccators until the analysis (Senofonte *et al.*, 1989). The samples were then treated according to the following procedure (Caroli *et al.*, 1992): 0.5 g of hair was cut into pieces no longer than 1 cm, thoroughly washed with a mixture of ethyl ether and acetone (3:1, v/v) under continuous stirring for 10 min, dried at 85°C for 1 h, and treated with a dilute (5%) aqueous solution of EDTA for 1 h. The pieces were repeatedly rinsed with double-distilled water and then dried at 85°C for 12 h in an oven to determine the dry weight of the sample just before the subsequent step. Hair digestion was based on irradiation with a microwave field at 2.45 GHz (Senofonte *et al.*, 1989; Caroli *et al.*, 1992). The treatment

steps were: overnight predigestion with 10 mL of high-purity concentrated (68%) HNO_3; 30 min stage at microwave power of ca. 180 W; 30 min stage at microwave power of ca. 240 W; 10 min cooling; addition of 1 mL H_2O_2 (30%) and 1 mL HF (50%); a further 30 min stage at microwave power 300 W; a 1 h stage at microwave power 360 W; quantitative transfer into polypropylene tubes; dilution up to 20 mL with double-distilled water. It should be emphasized that standardization of the methods of hair lead determination remains an unresolved problem (Furman & Laleli, 2000) and the washing method adopted to remove surface contaminants may produce large differences in PbH values (Lekouch *et al.*, 1999; Sen & Chaudhuri, 2001). The method used for quantification of Pb consists of inductively coupled plasma atomic emission spectrometry with a Jobin-Yvon 38+ Spectrometer. Qualitative control of the analysis of trace lead levels in hair was performed with the IMS 102 multielement standard of 10 µg/mL, certified for the calibration of the instrument by the Ultra Scientific Society, Bologna, Italy, and with BCR-397 (certified lead value of 33 mg/kg in 10 g of human hair, European Union Community Bureau of Reference).

From time to time, AAS measurements were also performed to check the reliability of the data.

Statistical analyses

The results of the Shapiro-Wilk W test indicated that all samples, with the sexes combined or separate, had a significantly non-normal distribution for PbB and PbH, except for the Portoscuso girls and the Sestu boys whose W test values for PbB were at the limit of significance (respectively: W=0.9559, p=0.0538; W=0.9359, p=0.0694).

Since the PbB and PbH distributions for both the combined and separate samples were non-normal, we calculated the values of the medians by interpolation of the cumulative distribution (Must *et al.*, 1991; Marascuilo & Serlin, 1998; Sanna *et al.*, 2000).

The correlation between PbB and PbH levels was tested with the Bravais-Pearson correlation coefficient using log-transformed values. Statistical analyses were performed with Statistica-Statsoft 6.0.

Portoscuso

The town of Portoscuso in south-western Sardinia (Sulcis-Iglesiente area), with 5,560 residents as of December 31, 1998, has been classified as urban (ISTAT, 1986). Since the 1960s, one of the most important industrial complexes on the island has developed in the area of Portovesme about 2 km from Portoscuso. It includes some factories for the refining and processing of aluminium, lead and zinc (Bodano & Dentoni, 1988; Cardia *et al.*, 1989; Melis & Senette, 1990; Giordano *et al.*, 1993).

The main industrial plants include:

- Euroallumina: production of aluminium oxide for electrolytic uses; average annual production 800,000 tons;
- Alluminia: primary and secondary aluminium products; average annual production 125,000 tons of primary product and 20,000 tons of secondary product;
- Comsal: production of aluminium bars and sheets; average annual production 22,000 tons;
- Portovesme srl (ex Enirisorse): production and marketing of non-ferrous metals and alloys (Pb, Zn, Cd and K_2SO_4);
- a thermoelectric plant with a 240 MW coal-fired group.

Among these plants, Portovesme srl produces the highest amount of lead emissions. This plant was constructed in the early 1970s to process Sardinian lead and zinc minerals (sulphides and oxides). In 1997, 115,490 tons of lead were produced.

Investigations carried out in Portoscuso in different periods have revealed environmental lead pollution: in soil and vegetables (Contu *et al.*, 1986); in wine (Melis & Senette, 1990); in wine, soil, grass, vegetables, milk and cheese

(Giordano *et al.*, 1993). A study conducted in 1997-1998 by the Department of Public Health, University of Cagliari, found that levels of metals (Al, As, Cn, Cu, Ni, Pb, Sb, Se, Zn) in the water supply of Portoscuso and of the Sulcis-Iglesiente area were below detectable levels (DISP, 1999). However, lead levels higher than those of control groups have been reported both in blood (Bodano & Dentoni, 1988; Cardia *et al.*, 1989; Floris *et al.*, 1995; Sanna *et al.*, 1995, 1999, 2000, 2002, 2003) and in hair (Sanna *et al.*, 2003).

Sant'Antioco

Sant'Antioco, a coastal centre on the homonymous island in south-western Sardinia, had 11,868 residents as of December 31, 1998. It has been classified as rural (ISTAT, 1986). Its traditional economy is based on agriculture and fishing. At present, tourism and especially the tertiary sector are also important.

As a result of industrial activities in the nearby industrial area of Portovesme, the towns of Portoscuso, Carbonia, Gonnesa, San Giovanni Suergiu and Sant'Antioco have been included in the zone of Sulcis-Iglesiente, defined as an "area of high risk of environmental crisis" on the basis of the Decree of the Italian Council of Ministers dated November 30, 1990.

Sestu

The town of Sestu, about 10 km from Cagliari (the capital of Sardinia), is situated in the area of the Campidano plain (southern Sardinia). It had 13,998 residents as of December 31, 1998, and has been classified as semi-urban (ISTAT, 1986). Owing to the characteristics of its economy and location, Sestu can be considered unexposed to lead pollution (Floris *et al.*, 1995; Sanna *et al.*, 1995, 1999, 2002, 2003, 2005).

Results

ANOVA showed that the differences in decimal age between the groups are not significant, even when they are subdivided by town and sex ($p \geq 0.05$).

Table 1 reports the mean and median for PbB and PbH in children grouped by town and sex. The boys of Portoscuso have the highest median value of PbB (10.86 µg/dL), followed by the girls of Portoscuso (7.24 µg/dL); they are followed, but with much lower values, by the boys of Sant'Antioco (4.22 µg/dL) and Sestu (4.06 µg/dL), and lastly by the girls of Sant'Antioco (3.50 µg/dL) and Sestu (3.39 µg/dL). There is a similar pattern for the PbH values: the Portoscuso boys have the highest median value (10.00 µg/g), followed by the Portoscuso girls (7.21 µg/g), Sant'Antioco boys (5.44 µg/g), Sant'Antioco girls (4.69 µg/g) and finally the Sestu boys (3.79 µg/g) and girls (1.56 µg/g).

Tab. 1 - Mean, and median of PbB (µg/dL) and PbH (µg/g) of the total sample and the sample divided by sex and town.

		Portoscuso				S. Antioco				Sestu			
Sex	Variable	N	Mean	SD	Median	N	Mean	SD	Median	N	Mean	SD	Median
M	PbB	53	11.30	4.01	10.86	41	4.51	1.72	4.22	32	4.09	1.25	4.06
	PbH		15.51	14.83	10.00		6.71	3.86	5.44		4.03	3.00	3.79
F	PbB	64	7.39	2.17	7.24	67	3.57	1.23	3.50	73	3.34	1.10	3.39
	PbH		8.82	7.01	7.21		4.99	2.80	4.69		2.83	2.24	1.56
M + F	PbB	117	9.16	3.68	8.41	108	3.93	1.50	3.70	105	3.57	1.19	3.57
	PbH		11.85	11.68	8.45		5.64	3.33	5.04		3.19	2.54	2.91

Table 2 reports the values of the Bravais-Pearson coefficient of correlation between decimal age, logPbB and logPbH for the children of the three Sardinian towns, with the sexes combined and separate. The values indicate a significant positive correlation between logPbB and logPbH when the sexes are combined (r=0.4351; p≤0.001) and considered separately (r=0.3989, p≤0.001, for males; r=0.3801, p≤0.001, for females). Instead, the correlations between blood lead and age and between hair lead and age are not significant either with the sexes combined or separate.

Discussion

The Bravais-Pearson coefficient of correlation for the total sample, with the sexes combined and separate, indicates a significant correlation between logPbB and logPbH. These results illustrate the significant degree of correlation between the two variables, although a high percentage of unexplained variance persists in the total sample (81.07%) and in males (84.09%) and females (85.55%) separately (Figures 1-3). The amount of unexplained variance could be due either to the limitations of hair as a biomarker of metal exposure (Renshaw , 1976; Ryabukin, 1978; Seidel *et al.*, 2001; Morton *et al.*, 2002; Barbosa *et al.*, 2005) or to the different sensitivities of the analytical techniques used to determine the lead levels in blood and hair.

It should be noted that when the data for Sestu, the town with the lowest PbB and PbH levels (Table 1), are removed from the sample, the values of the correlation coefficient between logPbB and logPbH are slightly lower for the males and for the sexes combined, with respect to the values for the total sample, whereas they remain virtually the same for the females (r=0.3305, p≤0.001, for males; r=0.3802, p≤0.001, for females; r=0.4135, p≤0.001 for sexes combined). This indicates that the degree of exposure does not affect the correlation between PbB and PbH.

Relatively low, but statistically significant, levels of correlation between PbB and PbH have been reported in Italian children living in Sassuolo (north-central Italy), a town with a high density of potteries. In a sample of 210 Sassuolo children aged 7 years, a significant correlation was found between lead in the blood and lead in the hair: r=0.125, p=0.005 (Bergomi *et al.*, 1989). Significant correlations, albeit with rather low r values, were also found between PbB and PbH in a sample of 158 Polish children (98 males and 60 females) of Miasteczko Šlaskia in Upper Silesia, Katowice province: total sample, r=0.270, p≤0.001; male sample: r=0.254, p≤0.05 (Chlopicka *et al.*, 1998). A significant correlation was also found between PbB and PbH in a sample of 189 children of the Russian city of Saratov: r_s=0.45, p≤0.05 (Esteban *et al.*, 1999).

Tab. 2 - Bravais-Pearson coefficient of correlation (r) between logPbB, logPbH and age for the children of the three Sardinian towns with the sexes combined and separate.

Sex	Variables	r
M	logPbB vs. logPbH	0.3989**
	logPbB vs. age	0.0678
	logPbH vs. age	-0.1197
F	logPbB vs. logPbH	0.3801**
	logPbB vs. age	-0.0178
	logPbH vs. age	0.0681
M+F	logPbB vs. logPbH	0.4351**
	logPbB vs. age	0.0168
	logPbH vs. age	-0.0111

** = p≤0.001

Conclusion

The results of the present study do not completely support the hypothesis that PbH values can be used as a substitute for blood lead levels. However, we can generally state that: 1)

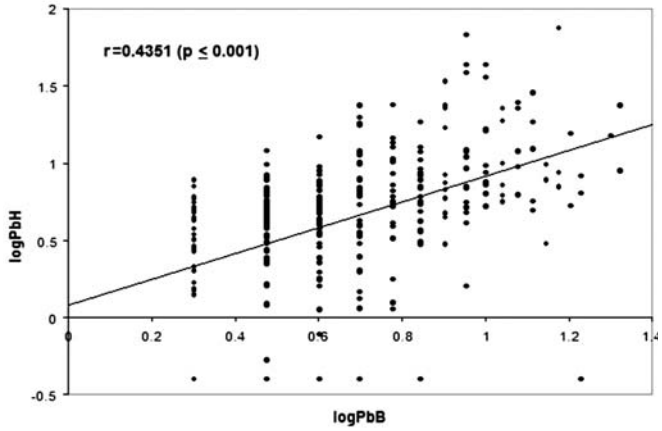

Fig. 1 - Scatterplot of logPbB vs. logPbH for the total sample of Sardinian children.

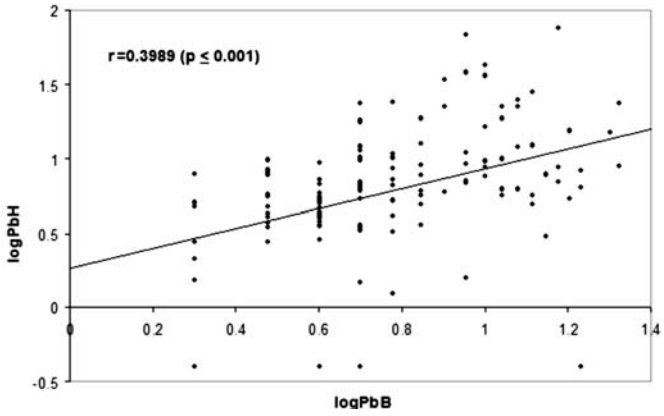

Fig. 2 - Scatterplot of logPbB vs. logPbH for the Sardinian boys.

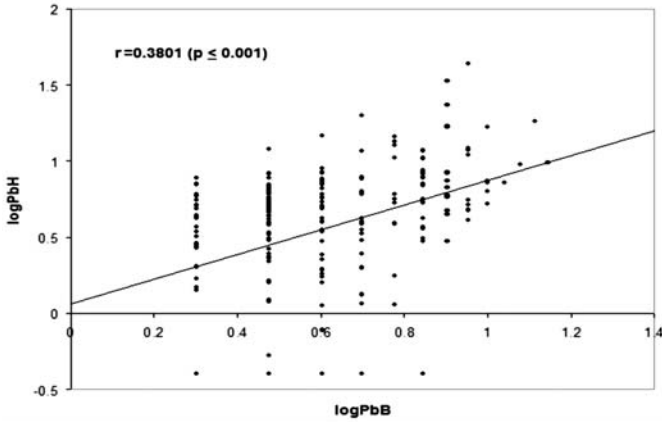

Fig. 3 - Scatterplot of logPbB vs. logPbH for the Sardinian girls.

the Portoscuso samples show higher PbB and PbH levels than the coeval samples of the other two Sardinian towns; 2) the boys present higher values of lead in the blood and hair than the girls of the same town. It is noteworthy that the correlations between logPbB and logPbH values, although statistically significant, present a high percentage of unexplained variance. Therefore, the determination of PbH can be used as a support for classical measurements of lead exposure but it cannot replace them. In fact, it can reveal a general pattern among samples with different environmental backgrounds but it cannot provide consistent results in the study of single individuals. Nevertheless, the pattern among samples with different environmental backgrounds and the significant correlations between the logPbB and logPbH values suggest that hair can be used as a suitable biomarker of lead exposure.

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