

# LEAD IN SALIVA AND ITS RELATIONSHIP TO BLOOD IN THE RESIDENTS OF KLITY VILLAGE IN THAILAND

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**Abstract.** Lead concentrations in whole blood and saliva were examined in 16 females and 13 males living in Klity village, a highly contaminated area from lead mining, Thailand. The geometric mean for the lead content in the blood was 24.03 µg/dl (range 11.80-46.60 µg/dl) while the lead content in the saliva was 5.69 µg/dl (range 1.82-25.28 µg/dl). No significant differences were found between the concentrations of lead in blood and saliva in relation to the age of the subject. Males were found to have higher blood lead levels than females. The coefficient of correlation  $\gamma$  between salivary and blood lead levels was -0.025. Our data suggests that saliva is not suitable material for biological monitoring with respect to lead exposure.

## INTRODUCTION

Lead has long been recognized as a serious environmental pollutant. It is one of the heavy metals that has many hazardous effects on the homeostasis of animals. Lead poisoning usually results from cumulative absorption of small amounts of lead until toxic concentrations are reached in the body. The severity of lead toxicity depends on the duration, frequency, and amount of exposure. When lead enters the blood it can cause serious health problems in adults. In children, however, the effects can be devastating. Children can sustain lead in their bodies for up to 17-20 years, thus destroying the central nervous system and causing chronic toxicity in the entire body (Goyer, 1996; Henretig, 1998). Developed countries have long realized the widespread detrimental effects of lead poisoning and have conducted extensive research and drawn up new and strong measures to control lead levels in air, water, and food. In Thailand, the problem of lead contaminated water in Klity Creek, Kanchanaburi Province was reported in April 1998 (Chaivisuth, 2002). The sources of lead are both natural and a result of lead min-

ing. Lead has been mined for more than 30 years in this area, and the untreated wastewater from the ore-dressing was discharged into Klity Creek. The Department of Pollution Control reported the estimate of the lead in the sediment of the creek was about 15,000 tons at a distance of 13 kilometers down stream from the creek (Chaivisuth, 2002; Rajesh, 2003). Since 1994, many village children in this area have been diagnosed with Down's syndrome and have had physical deformities, while the adults have suffered from an unidentified illness which caused the body to swell and ache. The first blood tests of the villagers in 1999 revealed that the lead concentration was 4-5 times higher than 4-9 µg/dl, the average for Thai adults (Rajesh, 2003).

Salivary monitoring has been used to monitor environmental pollutants, since circulating chemicals can be present in the salivary glands, which is reflected in the saliva. The concentration of these substances in the saliva depends on the nature of the material and its transport process. There are many reports indicating the distribution of lead in the salivary glands and its diffusion into the saliva in high concentrations (Craan *et al*, 1984; Mobarak and P'an, 1984). Using salivary sampling may be an alternative way to study lead exposure due to its non-invasive technique compared to venipuncture of blood. Therefore, the objective of this study was to evaluate the concentration of lead in saliva compared to the level in the blood.

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## MATERIALS AND METHODS

## Subjects

Twenty-nine subjects from both sexes (16 females, 13 males), from Klity Village, Kanchanaburi Province, Thailand, were recruited for this study. The average age of the subjects was  $28.75 \pm 15.03$  years (range 4-59).

## Sample collection

The subjects refrained from eating, drinking, smoking and oral hygiene for at least 2 hours before saliva collection. Paraffin stimulated whole saliva was collected in the morning under the same condition for each subject by the same examiner. All the saliva samples were collected in polypropylene sterile tubes over a single 5-minute period by expectoration. From each subject, 10 ml of peripheral blood was collected by venipuncture in a sterile tube. In the handling of samples, from collection and storage to analysis, great care was taken to prevent contamination. All containers used for collection and storage of samples were tested and found to be free from lead. All glassware used for analysis was washed thoroughly, rinsed with 10% nitric acid and then rinsed with deionized water.

## Lead analysis

Salivary and blood lead levels were determined using a graphite atomic absorption spectrophotometer (Varian Spectr AA 200, Victoria, Australia). Before analysis, samples were prepared by a microwave accelerated reaction apparatus (CEM Mar 5, Matthews, USA).

## Statistical analysis

Since the data displayed an abnormal frequency of distribution, it was necessary to ap-

ply the Komogorov-Smirnov test in order to get a distribution approximately log-normal. Geometric means, arithmetic means and ranges were calculated. Other data, such as age and sex, were also obtained. In the case of age, the study population was classified into two groups based on the arithmetic mean for the entire study population ( $\bar{x} = 28$ ; subject  $>28$  and subject  $\leq 28$ ). In relation to sex, code 0 was assigned to female subjects and code 1 to male subjects.

With the purpose of determining if there was any correlation between the blood and salivary concentrations of lead by age and sex, it was necessary to apply the non-parametric test of Wilcoxon-Mann Whitney. Goodman's test was used to assess the correlation between blood lead levels and salivary lead levels.

## RESULTS

Table 1 shows the blood and salivary lead means by age and sex. The geometric mean for the lead content in the blood was found to be  $24.03 \mu\text{g/dl}$  (range  $11.80-46.60 \mu\text{g/dl}$ ) while the mean level of lead in the saliva was  $5.69 \mu\text{g/dl}$  (range  $1.82-25.28 \mu\text{g/dl}$ ). There were no significant differences between the concentrations of lead in the blood and saliva in relation to the age of the subject ( $p > 0.05$ ). The concentration of lead in the blood was higher in males ( $28.01 \mu\text{g/dl}$ ) than in females ( $21.55 \mu\text{g/dl}$ ) ( $p < 0.05$ ). There was no significant difference in salivary lead levels between the sexes (female =  $5.51 \mu\text{g/dl}$ , male =  $5.92 \mu\text{g/dl}$ ). The mean salivary lead level was 23.67% of the blood, and the correlation between the lead levels in the saliva and the blood were poor ( $\gamma = -0.025$ ).

Table 1  
Salivary and blood lead concentrations ( $\mu\text{g/dl}$ ) by age and sex.

Classification		N	Blood <sup>a</sup>	Saliva <sup>a</sup>
Sex	Females	16	21.55 (11.80-39.15)	5.51 (1.82-14.93)
	Males	13	28.01 (16.9-46.6) <sup>b</sup>	5.92 (1.82-25.28)
Age	$\leq 28$	14	24.27 (13.50-36.14)	6.36 (1.82-25.28)
	$> 28$	15	23.81 (11.80-46.60)	5.16 (1.82-14.93)

<sup>a</sup>values are geometric means with the range in parenthesis

<sup>b</sup>significantly different from females

## DISCUSSION

The geometric mean for the blood lead levels in the normal healthy Thai population has been reported to be 3.25  $\mu\text{g}/\text{dl}$  (Sirivarasai *et al*, 2002). The lead levels in the blood of our group of subjects ( $\bar{x} = 24.03$ ) was found to be nearly 8 times higher than that of the normal population. Klity Village has been reported as having high levels of lead contamination, 75.9% of our subjects had blood lead levels  $\geq 25 \mu\text{g}/\text{dl}$ , which is defined as lead toxicity (Gerson, 1990; US Department of Health and Human Services, 2000). At this toxic level there is impaired heme synthesis, impaired red blood cell nucleotide metabolism, vitamin D and cortisol metabolism, and decreased intelligence. Signs of subclinical abnormalities may also occur. All the children ( $n = 8$ ), aged 3-6 years, had blood lead levels  $\geq 10 \mu\text{g}/\text{dl}$ , which is sufficient to cause adverse effects on cognitive development, growth, and behavior (National Research Council, 1993). Males had higher blood lead levels than females in our study. This is in agreement with a previous study in the normal healthy Thai population (Sirivarasai *et al*, 2002). The higher blood lead concentration in males than females has been suggested to be the result of occupational and environmental exposure and the effect of lifestyle factors, such as alcohol consumption and smoking (Rey *et al*, 1992; Watanabe *et al*, 1996; Sirivarasai *et al*, 2002). Since lead is a highly accumulative metal, which is preferentially concentrated in organ tissues and bone (Henretig, 1998), a relationship between blood lead levels and age has been observed by many researchers (Hense *et al*, 1992; Watanabe *et al*, 1996). However, our results, and the result of a previous study in the normal healthy Thai population, did not show this association (Sirivarasai *et al*, 2002).

Saliva has been suggested as a good monitor for recent lead exposure and is advantageous for use in screening programs, particularly in children (Cleymaet *et al*, 1991; Gonzalez *et al*, 1997). Salivary lead arises from the diffusible fraction of plasma and thus it represents the portion of circulating lead that is readily available for distribution to soft and hard tissues (Brodeur, 1983; Cleymaet *et al*, 1991). Using

saliva for biomonitoring is attractive because its collection is less invasive than venepuncture and is likely to be better accepted by individuals, especially for periodic collection. In addition, it is less costly, since a skilled technician is not required for collection. There are disadvantages to using saliva to determine lead levels. First, there is no standardized method for sampling saliva or determining salivary lead levels. There is no available external quality control. Second, there are no biological limit values for salivary lead in comparison to the well evaluated limits of blood lead. In earlier studies (P'an, 1981; Omokhodion and Cockford, 1991), subjects first rinsed their mouths with citric acid solution to stimulate salivary flow, followed by rinsing with deionized water, before collection of saliva. The method of saliva collection by Fung *et al* (1975) involved children chewing sugarless gum, which stimulates salivary flow prior to collection. In another study, parotid saliva was collected after reflex stimulation with orange flavored lozenges by a modified double-lumen Teflon-Lashley cup (DiGregorio *et al*, 1974). In our study, paraffin-stimulated saliva samples were collected. The results show that lead concentrations found in saliva were rather low (23.7% of blood levels), and their relationship to blood levels was poor. Nevertheless, salivary levels recorded in our study were higher than some previously reported values of 0.48  $\mu\text{g}/\text{dl}$  (Omokhodion and Cockford, 1991) and  $<0.15 \mu\text{g}/\text{dl}$  (Wilhem *et al*, 2002) due to our subjects living in a highly contaminated area. This finding is a good indication that heavy metal, lead in this case, is present in saliva in amounts much lower than in blood. The mean salivary lead levels recorded in our study are comparable to those reported by other workers, ranging from 13-56% of the mean blood lead levels (DiGregorio *et al*, 1974; Fung *et al*, 1975; P'an, 1981; Omokhodion and Cockford, 1991; Koh *et al*, 2003). P'an (1981) reported a good correlation between salivary lead levels and blood lead levels, however, some studies showed a weak correlation (Omokhodion and Cockford, 1991; Wilhem *et al*, 2002; Koh *et al*, 2003). One reason is that salivary lead levels may be proportional to the diffusible component of the plasma lead level rather than the whole blood

lead level. Secondly, there may be differential rates of excretion of lead into the saliva at different blood lead levels. Finally, the contamination of saliva during collection may be a potential problem.

In conclusion, lead concentrations recorded in the present study were higher than those previously reported in the normal healthy population due to the living in a highly contaminated area. There were no age-based differences between the blood and saliva lead levels. Males were found to have higher blood lead levels than females. Our data suggest that saliva is not suitable material for biological monitoring with respect to lead exposure.

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