Saliva as a Diagnostic Fluid in Lead Exposed Subject

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Abstract

***Background*:** Lead poisoning has been recognized since antiquity. In the second century B.C., Dioscorides, a Greek physician said "lead makes the mind give way" lead poisoning in adults continues to occur today, mostly as a consequence of occupational exposures. Heavy metal poisoning is the toxic accumulation of heavy metals in the soft tissues of the body.

In human, lead can results in a wide range of biological effects depending upon the level and duration of exposure. Effects at the subcellular level as well as effects on the overall functioning of the body have been noted and range from inhibition of enzymes to the production of marked morphological changes and death. Such changes occur over a broad ranges of doses, the developing human generally being more sensitive than the adult.

Objectives: To Study the possibility of using salivary lead concentration as alternative to blood lead concentration and estimate (clinical, biochemical, hematological and saliva cytological) changes to in lead exposed individual.

***Patients and Methods*:** The sample population of this study was comprised 56 lead exposed subjects and 20 healthy subjects. The general information's were taken from each person including the name, age and duration of the lead exposure as well as saliva and blood samples.

***Results*:** The result of this study showed a significant difference in the salivary lead concentration of the exposed and healthy groups while the blood lead

concentration in the same groups were highly significant difference, that is mean the saliva has a significant value in the detection of lead toxicity.

***Conclusion*:** Saliva testing for lead can become a valuable strategy for meeting the increasing demand for lead testing.

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Introduction

Many toxins and their metabolites are excreted in saliva, and salivary levels can reflect their levels in other body compartments [1].

The salivary gland represents a clearance organ for lead in blood stream. The half-life of lead in the saliva is much less than that of blood [2,3], suggesting a rapid removal of the PbS by various organs in the oral cavity especially the teeth, gingiva and tongue[4].

Saliva represents an easily accessible and useful body fluid for biomonitoring human exposure to environmental contaminants. Sialochemists point to several advantages of saliva over blood collection: it's non –invasive, so patients are spared the discomfiture of repeated venipunctures, it is the technique for children and patients with limited coping abilities. It is not coasty since there is no special training schedule for the staff and the patients can collect samples themselves, also there is no risk of infection or thrombosis and sample do not require special handling or preservation if they are to be used for trace metal analysis [5 ,6] . More importantly, the lead concentration in the saliva is closely related to the unbound plasma fraction and intracellular level and thus reflects the internal lead level that can exert effects on human organs [7, 8.]

Materials And Methods

Oral Examination

Oral examination was done using sterile mirror and a portable light torch. Oral findings were recorded in term of present or absent which include: lead line and ulceration.

Examination of Physical Properties of Saliva:

Saliva collection:

Unstimulated saliva samples were collected between 9-10 a.m.

Subjects were asked to refrain from eating, drinking, smoking, or oral hygiene procedures for at least 1hour before the collection.

Each subject was instructed to wash and rinse his/her mouth with water several times to insure the removal of any possible food debris and contaminating materials and asked to accumulate saliva in their mouth by spitting method, he/she spitted the saliva into graduated glass tube, after estimating the PH value, saliva sample then centrifuged at 4500 r.p.m. for 15 minutes.

After centrifugation the flow rate was measured and then the supernatant aspirated and put in a plain tube for quantitative analysis of lead, while the deposit was smeared on glass slide which was put in 95% ethanol alcohol for fixation of the cell to be stained by pap stain after that five field were examined at high power field (400x) for the sake of counting inflammatory cells, namely neutrophils, lymphocytes, monocytes, eosinophils, basophils and plasma cells.

Blood Samples Collection

Five milliliters of venous blood were collected by a venipuncture using disposable dry plastic syringe.

Measurement of Lead Level in Blood and Saliva:

1. The whole blood and the supernatant of the centrifuged saliva were used for quantitative analysis of lead by atomic absorption spectrometry device (Buck MODEL 210 VGP) in poising consultation centre.

Results

Table 1. Frequency distribution of lead exposed group

	Study group		
	Healthy controls	Exposed to Pb	P (t-test)
	N=20	N=56	
Salivary Pb concentration (ug/dl)			<0.001
Range	(2 - 4.5)	(3.5 - 10)	
Mean	3.2	5.3	
±SD	0.6	1.7	
±SE	0.14	0.23	
Blood Pb concentration (ug/dl)			<0.001
Range	(8 - 22)	(15 - 54)	
Mean	15.1	27	
±SD	4.6	11.2	
±SE	1.02	1.5	

According to salivary Pb concentration the mean concentration of healthy control and lead exposed groups were 3.2µg/dl, and with a highly significant differences ($p<0.001$).

Table 2. Among Exposed group only

	Toxic Blood Pb level (≥ 25 µg/dl)		
	Non-toxic (n=30)	Toxic (n=26)	P (t-test)
Salivary Pb concentration (ug/dl)			<0.001
Range	(3.5 - 7.5)	(4 - 10)	
Mean	4.6	6.2	
±SD	0.9	2.1	
±SE	0.16	0.41	
Salivary Inflammatory cells count			0.04
Range	(1 - 34)	(5 - 39)	
Mean	14.6	19.7	
±SD	8	10	
±SE	1.46	1.96	
Salivary flow rate (ml/min)			0.78 *[NS]*
Range	(0.15 - 1.5)	(0.1 - 1)	
Mean	0.43	0.46	
±SD	0.28	0.28	
±SE	0.051	0.054	
Salivary PH			0.92 *[NS]*
Range	(5 - 9)	(5 - 9)	
Mean	6.8	6.8	
±SD	0.9	1.1	
±SE	0.16	0.21	

According to salivary inflammatory cells count, the mean of cells count of non toxic and toxic subgroups were 14.6cell/HPF (range 1-34)cell/HPF and 19.7cell/HPF (range 5-39)cell/HPF respectively with significant differences (p-0.04).

According to salivary flow rate and salivary PH both parameters have no significant differences (p=0.78 and p=0.92) respectively.

Table 3. The rate of lead toxicity by selected parameters

	Total	Non Toxic Blood Pb level (<25 µg/dl)	Toxic Blood Pb level (≥25 µg/dl)		
	N	N	N	%	P
Ulceration					0.33[NS]
Negative	52	29	23	44.2	
Positive	4	1	3	75	
Lead line					0.13[NS]
Negative	40	24	16	40	
Positive	16	6	10	62.5	

Both oral ulceration and lead line show no significant difference between these two groups.

Table 4. Validity parameters of selected signs in diagnosis of lead toxicity.

	Toxic Blood Pb level (≥ 25 µg/dl)			Sensitivity	Specificity	Accuracy	PPV at pretest probability = 50%	NPV at pretest probability = 10%
	Non-toxic	Toxic	Total					
Ulceration								
Negative	49	23	72	11.5	98.0	68.4	85.2	90.9
Positive	1	3	4					
Lead line								
Negative	44	16	60	38.5	88.0	71.1	76.2	92.8
Positive	6	10	16					

- Ulceration: the sensitivity was 11.5, specificity 98.0 and accuracy 68.4.
- Lead line: the sensitivity was 38.5, specificity 88.0 and accuracy 71.1.

Table 5. Linear correlation coefficient of salivary and blood lead level with selected parameters

Pearson's linear correlation coefficient	Salivary Pb concentration (µg/dl)	Blood Pb concentration (µg/dl)
Salivary Pb concentration (ug/dl)		0.58(**)
Blood Pb concentration (ug/dl)	0.58(**)	
Age in years	0.37(**)	0.27(*)
Salivary flow rate (ml/min)	0.05	0.00
Salivary PH	-0.05	-0.04
Salivary Inflammatory cells count	0.12	0.32(**)

There was moderately strong linear correlation between (blood Pb concentration and salivary Pb concentration 0.58), (salivary lead concentration and age in years 0.37), and (blood lead concentration and salivary inflammatory cells count 0.32).

Table 6. Validity parameters for selected cut-off values of salivary lead concentration when used to diagnose lead toxicity.

Salivary Pb concentration (µg/dl)-Positive if ≥ Cut-off value	Sensitivity	Specificity	Accuracy	PPV at pretest probability = 50%	PPV at pretest probability = 90%	NPV at pretest probability = 10%
2.3	*100.0*	*2.0*	*35.5*	*50.5*	*90.2*	*100.0*
2.8	100.0	12.0	42.1	53.2	91.1	100.0
3.3	100.0	22.0	48.7	56.2	92.0	100.0
3.8	100.0	42.0	61.8	63.3	93.9	100.0
4.3	76.9	60.0	65.8	65.8	94.5	95.9
4.8	61.5	82.0	75.0	77.4	96.9	95.0
5.3	50.0	92.0	77.6	86.2	98.3	94.3
5.8	*50.0*	*96.0*	*80.3*	*92.6*	*99.1*	*94.5*
6.3	46.2	96.0	79.0	92.0	99.0	94.1
6.8	38.5	98.0	77.6	95.1	99.4	93.5
7.3	26.9	98.0	73.7	93.1	99.2	92.3
8.3	*19.2*	*100.0*	*72.4*	*100.0*	*100.0*	*91.8*

The typical cut-off value of salivary Pb concentration is 5.8µg/dl at this concentration the sensitivity was 50.0, the specificity 96.0 and the accuracy 80.3.

Discussion

The result of this study showed a significant difference in the salivary lead concentration of the exposed and healthy groups similar to the blood lead concentration, that means the saliva has a significant value in the detection of lead toxicity, This is in agreement with [2,9,10,11] who found that Lead is present in the saliva of lead exposed and unexposed individuals, and the significant correlations that have been reported with PbB suggest that saliva may be a valuable tool for assessing lead exposure also this is in accordance with [1,2] who found the same findings so that the saliva was a potential as a technique for monitoring ambient pollutants recent exposure, since circulating levels of certain polluting chemicals can be transported into salivary glands and secretions.

In comparison to recent studies the results of this study were higher than the other studies. This could be due to that the workers didn't use the preventive measures which include health education, using proper personal protective equipment with good personal hygiene.

In the present study there was a moderately strong linear correlation between blood Pb concentration and salivary Pb concentration (r=0.58).The same observation was carried out by [13] whom showed a strong correlation (r=0.41) among occupationally exposed individuals with high PbB values .Additionally this results is in agreement with Pan (1981) who found a good relation between PbB and PbSa (r=0.72) for adult male occupationally exposed to lead.

On the other hand our result is in disagreement with [14] who found a negative correlation between the two parameters and [15] reported that saliva is not a suitable material for biological monitoring with respect to lead exposure. These controversial findings among the authors concerning the validity of saliva as a tool for monitoring Pb toxicity may be attributed to variation in the assessment methods, variation in sample size and variation in lead concentration additionally saliva shows large variations in its ion content throughout the day, coupled with changes in salivary flow rates before, during, and after meals.

Clearly, in this research there is a significant difference of salivary inflammatory cells count between toxic and non-toxic groups (P=0.04) also there is moderately strong linear correlation between salivary inflammatory cells count and blood lead concentration (r= 0.32), this change might be due to lead-induced inflammation.

Also there is a statistically moderately strong linear correlation between salivary Pb concentration with age in years (r=0.37). This observation suggests that the saliva may be an important route of excretion of lead by elderly adults[17]. In contrast to these observations, Barbosa et al (2006) found that the age does not affect Pb saliva levels.

Clearly, the upper acceptable limit of saliva Pb is a matter of controversy . From this study the cut-off point of saliva lead level was 5.8µg/dl.

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