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Predicting Blood Lead Levels Using Endogenous Lead Levels in the Scalp Hair of Workers in Acid Battery Manufacturing Plant: A Pilot Study

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Abstract

Twelve hair samples were collected from a selected representative worker group working in a battery plant. The hair samples were analyzed for lead (Pb) levels using Atomic Absorption Spectrophotometry (AAS). Blood lead (BPb) levels were predicted from the results of hair samples lead levels following application of Clayton and Wooler (1983) model. The arithmetic mean for both endogenous Pb levels and predicted BPb levels were 8.95 ± 7.66 mg/g and 85.26 ± 22.57 µg/100 mL respectively. The pearson correlation coefficient between endogenous Pb levels and predicted BPb levels was (r = 0.88). The arithmetic mean BPb level was above the internationally reported acceptable level. Endogenous Pb level in scalp of hair samples is considered as a good specimen for predicting BPb level.

Keywords: Blood lead level, prediction, hair, battery plant, biomarker

1. Introduction

Lead (Pb) poisoning is considered a significant environmental disease (Wani et al., 2015). Its effects on the human body are deleterious, which appears in damaging the cardiovascular system, hematopoietic system, nervous system, kidneys, and reproductive system (Needleman et al., 1996).

Occupational exposure is believed to be the major cause of Pb poisoning in adults, as manifested in workers of battery plants (Qasim and Baloch, 2014). Approximately, 85% of the total global consumption of Pb is for Pb-acid batteries production (ILA, 2017). In many countries, acid battery manufacture exposure is almost unregulated, and monitoring of exposure rarely exists (Manhart et al., 2016). Therefore, Pb fumes and dust that are generated from such operations pose an exceptional health risk not only to the workers inside the factories but also to individuals living near these operations (Rodney, 1985).

Although BPb Level is considered the dominant biological marker used in workplace monitoring and clinical assessment regarding removal from exposure (Qasim and Baloch, 2014), other biological samples like hair, breast milk, nails, and urine had usually been used for trace elements, including Pb, determination in human body become very important. Hair has many advantages for its easy and painlessly collection, easy transport, storage, and low cost (Dombovári and Papp, 1998). Unlike blood, hair is less vulnerable to reactive homeostatic mechanisms that affect trace element levels (NBO, 2020).

The validity of using hair sample as alternative to blood sample in determination of Pb levels in workers occupationally exposed should be examined. In Jordan, there are many acid battery manufacturing plants which are considered as a potential major source of Pb emissions and pollution (Nsheiwat et al., 2010). The main aim of this study was to determine endogenous hair Pb level as a marker of lead exposure in workers of acid battery manufacturing plant located in Marka, Jordan. The obtained results were applied to evaluate the validity of using endogenous Pb levels in predicting BPb levels in same workers.

2. Materials and methods

2.1. Chemicals

Lead free concentrated nitric acid (HNO_3, 69-71%) of spectroscopic grade was obtained from Gainland chemical factory, UK. Acetone (99.6%) of spectroscopic grade was purchased from Fisher scientific. Lead standard solution 1000 ppm was obtained from BDH laboratory supplies, UK. Deionized water from analytical reagent grade water purification system (Millipore) was used.

2.2. Sample collection and preparation

All laboratory equipment used for the sample collection and sample treatment were cleaned and soaked with 10% nitric acid overnight, rinsed using deionized water, then dried with acetone.

Hair samples were collected from twelve different healthy adult Jordanian males working in acid battery plant that is located in Marka (north eastern part of Amman). Hair was cut from different zones of the scalp (Feisal et

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al., 2019). Each hair sample was cut into pieces as small as possible and mixed to allow representative sub-sampling. 200 mg of each hair sample was accurately weighed and stored in polyethylene bags at room temperature until analysis. Also, seven control hair samples were collected from seven different persons in a non-contaminated area.

2.3. Sample wash

200 mg of each hair sample was washed and agitated two times with 20 mL deionized water into 250 ml Erlenmeyer flask (Krueger and Duguay, 1989). Contact time of the distilled water with the hair sample was 20 min each time. The two volumes of deionized water wash were combined into separated labeled 50 ml volumetric flask and preserved for further analysis for determination of exogenous lead. Washed hair samples were dried at 110 ºC and kept for determination of endogenous lead.

2.4. Sample digestion for exogenous Pb determination

Each wash of hair sample, separated in 50 mL, was transferred into individual clean 100 mL beaker. After that, each beaker was heated to around 100 ^{o}C on a hot plate under a fume-hood until most of the water was evaporated. Then, 10 mL of concentrated nitric acid was added to each beaker and covered with a watch glass. Each beaker was heated to approximately 120 ^{o}C until most of the liquid was evaporated. Additional 5 mL of nitric acid were added to each beaker and digestion continued until the digests were clear and about 2-3 mL of acid remained. The digests were cooled for 1 hour, diluted to 50 mL with deionized water and kept for analysis by AAS.

2.5. Sample digestion for endogenous Pb determination

Each washed hair sample was transferred into individual clean 100 mL clean beaker. 10 mL of concentrated nitric acid was added to each beaker and covered with a watch glass. The beakers were heated to approximately 120 ^{o}C on the hot plate under a fume-hood until most of the liquid was evaporated. Another 5 ml of concentrated nitric acid was added to each beaker and digestion continued until the digests were clear and 2-3mL of acid remained. After that, the digest was cooled for 1 hour, diluted to 50 mL with deionized water, and kept for analysis by AAS.

2.6. Prediction of BPb levels in acid battery workers

Prediction of BPb levels for workers of the current study was performed using their endogenous hair Pb levels, by applying the model developed by Clayton and Wooler (1983).

2.7. Instrument working conditions

Atomic absorption spectrophotometer with flame atomization (SpectrAA. 250 plus Varian), (department of chemistry, faculty of science, the University of Jordan) was used (Al-Subeihi, 2002).

2.8. Quality Assurance and Control

Determination of Pb levels in hair samples was performed based on the analysis method validated by Al-Subeihi (2002). Reagent blank samples were digested similar to hair samples and were used to correct instrument readings. Analysis of blank samples revealed no detection for any trace of Pb. Accuracy and precision of the method were validated by six replicate measurements using spiked hair samples at concentrations of 5 ppm and 20 ppm (Table 1) (Al-Subeihi, 2002).

Table 1. Accuracy and precision results (Al-Subeihi, 2002)

Spiked Pb (µg/ mL)	*No. of samples	Measured Pb (µg/mL)	Accuracy (Relative error)	Precision (STDEV)
5	6	4.35	13	0.124
20	6	19.14	4.3	0.886

2.9. Data analysis

Since the size of measurements in this study was small ($n < 30$), the normality of the data had been assessed appropriately using Shapiro-Wilk test using SPSS. The one sample t-test (one tailed) was used to test whether the sample mean of the study group is significantly different from a reference value of non-contaminated group. Results were considered significantly different when $p < 0.05$.

3. Results

3.1. Determination of endogenous Pb in hair samples

The mean level of Pb in hair samples of workers was 8.95 ± 7.66 mg/g, and ranged from 0.26 to 22.63 mg/g (Table 2). On the other hand, The mean Pb level in hair samples obtained from persons living in a non-contaminated area was < 0.2 µg/g. The Shapiro-Wilk test result ($p = 0.217$) indicated that the sample came from a normal population. A one sample t-test (one tailed) between the reference value obtained for endogenous Pb levels in scalp hair of individuals of the non-contaminated area (< 0.2 µg/g) and the mean level of Pb of the battery workers indicated that there was a significant difference between the two groups ($p = 0.001$).

Table 2: Predicted BPb Levels

Sample no.	Endogenous Pb (mg/g)	Exogenous Pb (mg/g)	Predicted BPb levels (µg/100 mL)
1	0.70	32.63	57.16
2	22.63	0.12	109.04
3	9.32	5.57	95.80
4	2.71	0.90	77.36
5	8.56	0.19	94.53
6	4.65	5.81	85.42
7	0.26	0.91	42.38
8	0.56	8.36	53.83
9	13.33	10.34	101.14
10	21.23	8.80	108.09
11	12.60	9.47	100.30
12	10.82	0.314	98.03
Mean	**8.95**	**6.95**	**85.26**
STD	**7.66**	**9.02**	**22.57**

3.2. Prediction of BPb levels in acid battery workers

Clayton and Wooler (1983) measured BPb and hair Pb levels of 38 male workers at a Pb acid battery plant in Sydney (Clayton and Wooler, 1983). The correlation between BPb and hair Pb levels in the study of Clayton and Wooler was significant: $r = 0.76$. The data followed an exponential curve: $Hair\ Pb\ level = 15.2e^{0.067 * BPb\ level}$

where the BPb level is expressed as µg/100 mL and the hair Pb level is expressed as µg/g (Clayton and Wooler, 1983).

BPb levels in acid battery plant workers of this study were predicted by extrapolation, using Clayton and Wooler (1983), the results ranged from 42.37 to 109.04 µg/100 mL (mean of 85.26 ± 22.57 μg/100 mL). The result of Shapiro-Wilk test ($p = 0.06$) suggested that the sample came from a normally distributed population. According to the Occupational Safety and Health Administration (OSHA, 1991), BPb levels of 40 µg/100 mL or more suggest possible Pb toxicity. A one sample t-test (one tailed) indicated a significant difference between the reference value of OSHA and the mean value of workers of the acid battery plant ($p < 0.0005$).

3.3. Quality Assurance and Control

Analysis of blank samples revealed no detection for any trace of Pb. Accuracy and precision of the method were validated by sex replicate measurements using spiked hair samples at concentrations of 5 ppm and 20 ppm (Table 1). The results of the observed Pb concentration were in excellent agreement with the certified values and the method recoveries were 87 ± 2.9 % and 95.7 ± 4.6 %, respectively (Al-Subeihi, 2002).

4. Discussion

Pb intoxication is common among persons chronically exposed to high Pb levels. Occupational exposure is the main cause of Pb poisoning in adults, as in Pb miners, battery workers, smelters, plumbers, glass manufacturers, cosmetic workers, etc.

In Jordan, the main source of occupational Pb toxicity is acid battery manufacturing plant. This pilot study aimed at measuring endogenous hair Pb level as a marker of Pb exposure in workers of acid battery manufacturing plant located in Marka, Jordan. The obtained results were applied to evaluate the validity of using endogenous Pb levels in predicting BPb levels in the same workers.

The average of endogenous Pb level in hair samples of workers was 8.95 ± 7.66 mg/g, and ranged from 0.26 to 22.63 mg/g (Table 2). The variation in endogenous Pb levels might be justified in that some individuals worked for long periods in the battery plant than others, which revealed a long term exposure to Pb. On the other hand, Pb in hair samples obtained from persons living in a non-contaminated area was < 0.5 mg/g. The results obtained for endogenous Pb in hair revealed high deposition levels of Pb. It was concluded that occupational exposure to Pb is reflected by elevated levels of scalp hair Pb of exposed humans in polluted areas.

It was reported that Pb level in scalp hair of some workers of Pb battery plant was 2.3 mg/g (Noguchi et al., 2014), where the average Pb hair levels of the current study was 3.9 fold higher. In one study, the average Pb hair level was about 0.363 mg/g (Fergusson et al., 1981). It was noticeable that the present findings were around 25 fold more than that reported by Fergusson et al. Elevation of endogenous Pb levels in scalp hair may demonstrate long term exposure to Pb in the acid battery plant.

Washing procedure using de-ionized water was sufficient enough to remove the deposited Pb. This was inferred by noting the results summarized in Table 2. For example, analyzing the wash of scalp hair in samples (1, 6, and 8) to measure the exogenous Pb level revealed high Pb levels whereas the endogenous Pb levels were low. The variation in exogenous Pb levels as shown in table 2 might be justified in that size of work inside the battery plant differs from one day to another. Due to this difference, different amounts of Pb had been released to the atmosphere of the plant and cause different deposition of Pb on the outer surface of workers scalp hair.

BPb levels in acid battery plant workers of this study were predicted by extrapolation, using Clayton and Wooler (1983); the results ranged from 42.37 to 109.04 µg/100 mL (mean of 85.26 ± 22.57 μg/100 mL). Strong correlation is expected between endogenous Pb levels in scalp hair and the predicted BPb levels, and the correlation is r=.88. This correlation supported using Pb level in scalp hair to predict BPb level in exposed individuals. These predicted results were comparable with the results of workers involved in Pb acid battery plant in Bangladesh (78.70 μg/100 mL) (Ahmad et al., 2014). Also, a mean BPb level of 96.70 ± 27.90 μg/100 mL had been reported in a study performed in Tehran, Iran (Reihani and Niktab, 2005).

According to OSHA, BPb level should be below 40 μg/dL where levels more than 40 μg/100 mL, worker should be informed in writing and provided with medical examination. Moreover, workers having BPb level of 60 μg/100 mL for a single time must be removed from their job and could be placed to a job of lower exposure (OSHA, 2018). Workers with BPb level above 60 μg/100 mL commonly suffer from chronic nephropathy which may progress to kidney failure (Gidlow, 2007). All predicted BPb levels in the workers of the current study are greater than 40 μg/dL and around 75% were greater than 60 μg/100 mL while 66.7% are greater than 80 μg/100 mL (Table 2). Workers with BPb levels of 40 μg/100 mL or more suggest possible intoxication with Pb and willingness to show the symptoms of Pb poisoning (OSHA, 1991).

Qasim and Baloch (2014) reported 2 cases of occupational Pb poisoning in adult battery workers where BPb levels in case 1 and case 2 were 98.83 and 120.20 μg/100 mL, respectively. Both male patients had initial non-specific symptoms of intermittent abdominal pain, fatigue and headache for 6 - 8 years. Later on, they developed psychosis, slurred speech, tremors of hands and initially underwent treatment for Parkinsonism and Wilson's disease because of clinical misdiagnosis. Therefore, it could be concluded that the workers of the acid battery plant were chronically exposed to Pb and were at the risk of developing Pb toxicity.

5. Conclusion

Pb concentrations in hair wash and endogenous Pb levels in hair of battery plant workers were relatively high compared to control hair samples collected from seven different persons in a non-contaminated area. It could be concluded that workers of battery plant are at high risk for Pb pollution.

Strong correlation is expected between endogenous Pb levels in scalp hair and the predicted BPb levels and the correlation is $r = 0.88$. This correlation supported using Pb level in scalp hair to predict BPb level in exposed individuals.

6. Recommendation

Based on these results and conclusions, means to reduce exposure are highly recommended including reduction of air Pb concentrations. Moreover, monitoring of BPb levels among workers is strongly suggested. Also, educational programs of various workers group on the health and effects Pb should be held regularly.

Conflict of Interest

None to Declare.

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