

Heavy metals in blood and urine impact on the woman fertility

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Abstract

This study was designed to investigate heavy metals that were associated with fertility of women. Heavy metals have been identified as factors affecting human fertility. For a lot of women, whole blood specimens were analyzed for Pb, Zn, As, Mg, Co, Sn, Ag, Sb, Fe, and Cr using ICP-MS. Heavy metals as estimated has an impact on the hormonal profile and fertility in women. Findings indicate that Fe, Mg and Zn may impact female fecundity, also findings indicate that Fe, Mg and Zn caucus a different affects in the blood and urine samples , Fe >Mg> Zn > Pb > Sn > Cr as shown by the high concentrations of Fe, Mg, Pb, and Zn, in the blood and in urine samples. Fe >Mg> Zn > Pb > Sn > Cr as shown by the high concentrations of Fe, Mg, Pb, and Zn in the blood and in urine samples. Significant difference($p < 0.001$) found in blood concentration of these metals, where highest concentration was found in Fe, while the lowest observed in Cr, also showed significant ($p < 0.001$) difference among the concentrations of these metals in urine. The highest concentration was noticed in Mg, while the lowest was in Cr,

Keyword: Heave metals, blood, urine, lead, women infertility

1. Introduction

Toxic metals have been studied for centuries. The general population is exposed to lead (Pb) mainly from petrol, industrial emissions, paint and ceramics. Therefore, for risk assessment, there is a need for adequate information about concentrations of heavy metals in the liquids of the body for human such as blood and urine (Stasys, 2007). The heavy metals are required by the body in small amounts, but can be toxic in larger doses Humans are exposed to heavy (metals through inhalation of air pollutants, consumption of contaminated drinking water, exposure to contaminated soils or industrial waste, or consumption of contaminated food. Food sources such as vegetables, grains, fruits, fish, and shellfish can become contaminated by accumulating metals from surrounding soil and water.

Environmental factors such as metals may interfere with reproduction in women escipially lead Pb is widely distributed in the environment and are recognized reproductive toxicants. Exposure to high concentrations of trace metals may diminish fecundity, defined as the biologic capacity for reproduction .Several metals including (As, Cd, Mg, and Zn) have been detected in human follicular fluid (Blooma, 2011). Different metals present in the composition of blood can form different complexes with many organic compounds and bimolecular which could be found in the body fluids. Depending on the concentration of metals in the parts of body, different metal. The aim of the present study was to investigate, for the first time the distribution of heavy metals between cells in the blood samples from the infected by women infertility or weakness in fertility. The levels of metals in the blood and urine were influenced by several factors for example time exposure to the heavy metals, the quantity of metals, the types of metals and how the toxic metals affects the body. Heavy metals may occur in differently bound fractions in the blood and urine, (Iman, 2011). Coupled Plasma Mass Spectrometry (ICP-MS) is the first time used in Malaysia to study heavy metals in blood and urine to determine the harmful effects on women fertility. Note: Some coagulation can occur during acidification, due to natural variations in human blood (Sekhar, 2009).

heavy metals must be determined at very low levels in biological fluids such as blood and urine. (which is usually). Heavy metal contamination from occupational origin is a cause for concern because of its potential accumulation in

the environment and in living organisms leading to long term toxic effects. Blood and urine samples are the most widely used and accepted matrices for biomonitoring heavy metal exposure in occupational and environmental toxicology (Fernando, 2011) Moreover, Blood Lead Levels (BLLs) as low as >10 ug/dL are linked to harmful consequences. Due to its lower detection capability, inductively coupled argon plasma mass spectrometry (ICP-MS) the preferred method for analyzing at 10 ug/dL (Karen, 2008).

2. Experimental

2. 1. Material and Method

● *Blood*

Blood is a circulating tissue composed of fluid plasma and cells (red blood cells, white blood cells, platelets). Anatomically, blood is considered as connective tissue because of its origin in the bones and its function. Blood, as the transport system of the body, transports elements (e.g., nutrients, waste products, and heat) from one location in the body to another via blood vessels. In healthy individuals, the distribution of blood cells is maintained within a strict range. However, illness, hemorrhage, radiation, and chemotherapy adversely affect blood cell counts (Alejandro, 2005) Whole blood is a more complex sample due to its higher levels of proteins and other organic compounds(Zhu et al., 2009).

● *Urine*

Urine is a liquid that is secreted by the kidneys through a process called urination. The kidneys extract the soluble wastes from the bloodstream, it filters in the kidneys. The density values of urine varies between 1.003-1.035 gcm^{-3} (Wikipedia, 2009).

2. 2. Method

All chemicals used were of analytical reagent grade and all solutions were prepared. Calibration standards of each metal were prepared by appropriate dilution of stock solution. Measurement of Metal Contents Concentrations of (Pb , Zn, As, Mg, Co, Sn, Ag, Sb, Fe, Cr) carried out, were measured in blood and urine by using ICP-MS shows in Figure1 and Table 1

2. 2. 1 Sample collection and preparation

● **Microwave machine analysis**

The samples of urine samples and venous blood samples were collected from the women had infertility and weakness fertility of limit age from 20 to 40 years (All women were medically fit and symptom-free at the time of this study) and analyzed for the heavy metal content using ICP-MS after collection, the blood and urine samples were placed in a ice cool box (4°C) and transferred to the laboratory for refrigeration until analysis. Samples were prepared following the method suggested by (Teresa, 2009) Before the carry out the process to reagents, the preparation of samples carried out by : (Teresa, 2009) samples of blood (2mL) , urine (2-8) mL with addition of 4 mL nitric acid (%65 HNO_3) and 1mL hydrogen peroxide (%30 H_2O_2) to both samples by using microwave system. The main advantages of microwave digestion are that it requires smaller amounts of sample and oxidizing materials, shorter digestion times, and easiness of sample handling. The present study was designed to evaluate the levels of heavy metals in blood and urine. Certified reference materials confirmed the validity of the analytical procedure (Ingrid et al., 1998) and used to change the color of blood and urine samples to the white color each cycle 35 mint for process,

● **Reagents and assessments by ICP-SM**

All calibration standard solutions, used in calibration the heavy metals in the samples, were prepared from 1 mg/ mL for 1 single element standard solutions by dilution with ultrapure water. Two different reference materials were used in this study: Trace elements urine, trace elements blood level 2, these reference materials were kept in the refrigerator at 4°C until analysis and were reconstituted. The operating conditions and measurement parameters.

3. Statistical analysis -SPSS

All statistical calculations were performed using SPSS for Windows, Standard version 20.0. The data of the research were saved in Microsoft Excel Spread sheet and analyzed on the computer using Microsoft Excel program used in this analysis Independent on non-parametric tests and Kruskal-Wallis test.

4. Result and discussion

Heavy metals have been identified as factors affecting human fertility. This study was designed to investigate heavy metal that is associated with different factors of infertility. Significant correlations were found between different heavy metals and biological samples, and thus may play a role in the increasing infertility problem, the hypothalamic-pituitary-ovarian axis can be affected by heavy metal so vary itself, accumulation of heavy metals impairs the production of estradiol and progesterone may interfere with the normal oocytic development and cause chromosomal damage. Heavy metals as estimated has an impact on the hormonal profile and fertility in women. Therefore, the prevalence of infertility has increased from 8 to 15% over the past 2 decades in industrialized countries (Tasneem et al., 2008)

The present study was to compare the level of essential trace elements, chromium (Cr), copper (Cu), iron (Fe), manganese (Mg), and many metals in biological samples in (whole blood, urine). Trace exposures to metals may affect female reproductive health. To assess the relation between trace concentrations of blood metals and female fecundity. For a lot of women, whole blood specimens were analyzed for Pb, Zn, As, Mg, Co, Sn, Ag, Sb, Fe, and Cr using inductively coupled plasma mass spectrometry (ICP-MS), shown in Tables 2, 3

Findings indicate that Fe, Mg and Zn may impact female fecundity, that is agree with result in the Ref. (Stasys, 2007) and compared the result with many results for many countries, shows in the Tables 4, 5.

Human exposure assessment revealed that the concentrations of Mg, Zn, Fe and Pb are much higher than the permissible levels in people residing in the study area that is also agree with the result in the Ref.(Stasys, 2007). It is very difficult to draw a correlation between metal content of blood and urine with age and number of patients. However, it is clear from results reported here that patients risk associated with exposure to metals pollution not depend on the number of them. This is the first time linked radon area monitoring with measured the toxic metals in blood and urine samples from the number of women weakness in the fertility. carried out evaluate the status of heavy metal exposure in the blood and urine samples from women and affects on the fertility of women, study found a significant correlation between infertility and blood lead levels in spite of their low concentrations. The wave length of heavy metals in the blood and urine samples were resulted in the Table 6

The level of Zn was low in whole blood. The heavy metals (Co, Ag, Sb) not registered any accept number in all samples (in blood and urine samples) because the concentrations are lower than the detection limit determined for these three elements. As expected, the concentration of Fe is much higher in comparison with other elements in the blood, but in urine the concentration of Mg is much higher in comparison with other elements, shows in the Figures 2, 3. The concentrations of the biologically important elements Mg and Sn concentrations in blood, also the concentrations of Zn were found to be higher than the Pb in urine because the soil of the area under study contain very toxic chemistry metals due to the competent war and bombarded the toxic chemistry metals in this area, and the geological component of this area also contain the heavy metals. Also Pb may impact female fecundity, but in varying directions. $Fe > Mg > Zn > Pb > Sn > Cr$ as shown by the high concentrations of Fe, Mg, Pb, and Zn, in the blood and in urine samples, shows in the Figures 2, 3.

The results of statistically showed, there were 43 experiments conducted for six metals to find out the differences among these metals in their concentration in blood. The mean (\pm SD) concentration among all these experiments was 5.374 ± 9.55 , while the median was 1.047. The concentrations of these metals were not normally distributed, thus non-parametric tests were used. Kruskal-Wallis test was the appropriate statistical test in order to find out the difference among results of these experiments. Significant difference found in blood concentration of these metals, where highest concentration was found in Fe, while the lowest observed in Cr, as shown in Table 7

There were 31 experiments were conducted for five metals to find out the differences among these metals in their concentration in urine. The mean (\pm SD) concentration of these metals in urine was 6.102 ± 12.476 , while the median was 0.106. Also non-parametric tests were used because the results of these experiments were not normally distributed. Kruskal- Wallis test showed significant difference among the concentrations of these metals in urine. The highest concentration was noticed in Mg, while the lowest was in Cr, as shown in Table 8.

Findings indicate that, Mg and Zn and exposures to metals may affect female reproductive health and impact female fecundity, but in varying directions Other metals, including magnesium (Mg), nickel (Ni), selenium (Se), and zinc (Zn) are essential for human reproduction in trace concentrations however, exposure to excess quantities of these elements may also be hazardous Several metals including As, Cd, Mg, Pb, and Zn have been detected in human follicular fluid underscoring the proximity of these agents to reproductive organs and tissues [Some coagulation can occur during acidification, due to natural variations in human blood (Sekhar, 2009). On the other hand, the mean average calibrated and measured blood pH was (7.50) and measured urine pH was (5.87).

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Figure1 The process of determination the concentration of heavy metals in blood and urine samples by (ICP-MS) instrument.

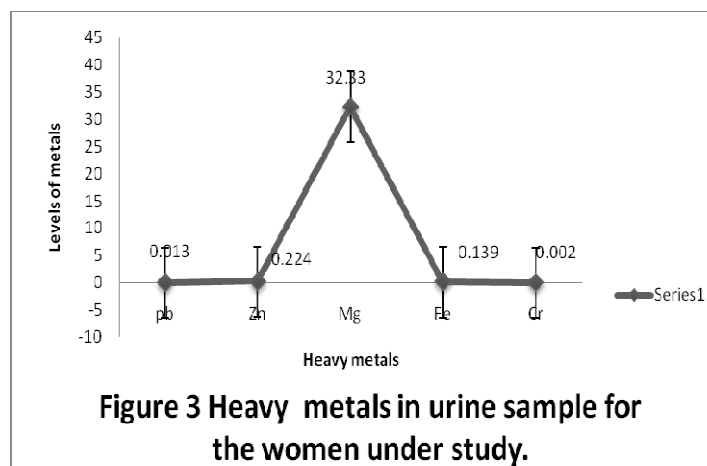
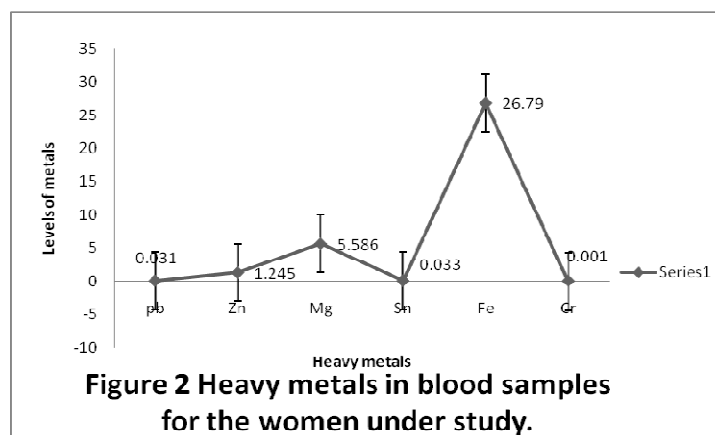


Table 1 The calibration of metals in the samples.

	Ag 328.068	As 193.696	Co 228.616	Cr 267.716	Fe 238.204	Mg 285.213
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Calib Blank 1						
Std 1						
std 2						
std 3						
1	-1.919	-0.022	-0.009	0.001	26.79	5.586
2	-1.423	-0.026	-0.009	0.003	34.43	3.815
3	-2.535	-0.023	-0.007	0.001	19.36	4.051
4	-1.390	-0.028	-0.008	-0.000	29.26	4.178
5	-2.440	-0.025	-0.009	-0.001	17.74	3.185
6	-2.455	-0.020	-0.009	-0.002	13.66	3.594
7	-2.418	-0.023	-0.009	-0.001	18.00	3.162
8	-1.232	-0.029	-0.009	-0.000	31.32	3.154
9	-1.494	-0.000	-0.007	0.002	0.139	32.33
10	-1.592	-0.009	-0.008	0.001	0.058	21.14
11	-1.550	-0.002	-0.007	0.005	0.077	28.08
12	-1.723	-0.012	-0.008	-0.002	0.098	42.02
13	-0.654	-0.012	-0.007	-0.004	0.130	19.72
14	-2.301	-0.004	-0.006	-0.005	0.072	36.78
15	-2.643	-0.001	-0.006	-0.011	0.012	1.627
16	-2.573	0.002	-0.006	-0.013	0.006	5.739

Table 2 The heavy metals concentration (mg/L) in blood samples collected from the women in the location under study

No.	Element (mg/L)					
	Pb	Zn	Mg	Sn	Fe	Cr
1	0.031±0.0017	1.245±0.0215	5.586±0.0099	0.033±0.0054	26.79±0.296	0.001±0.0006
2	0.027±0.0003	1.047±0.0068	3.815±0.1505	0.017±0.0012	34.43±0.018	0.003±0.0003
3	0.034±0.0016	1.428±0.0107	4.051±0.0293	0.011±0.0034	19.36±0.084	0.001±0.0001
4	0.256±0.0016	1.268±0.0192	4.178±0.0497	0.013±0.0003	29.26±0.117	ND
5	0.018±0.0010	0.928±0.0264	3.185±0.0709	0.008±0.0008	17.74±0.1210	ND
6	0.070±0.0024	0.802±0.0067	3.594±0.0372	0.005±0.0007	13.66±0.016	ND
7	0.063±0.0003	1.189±0.0182	3.162±0.0021	0.007±0.0006	18.000±0.255	ND
8	0.130±0.0010	1.159±0.0144	3.154±0.0234	0.004±0.0004	31.32±0.7190	ND
Ave.	0.0786	1.1332	3.8406	0.01225	23.82	0.0025
**	0.011±0.0011	0.561±0.0123	3.056±0.0211	0.003±0.0031	9.221±0.1012	ND

** Mean the normal woman

Table 3 The heavy metals concentration (mg/L) in urine samples collected from the women in the location under study.

No.	Element (mg/L)					
	Pb	Zn	As	Mg	Fe	Cr
1	0.013±0.0009	0.224±0.0008	ND	32.33±0.0220	0.139±0.0001	0.002±0.0005
2	0.062±0.0021	0.106±0.0012	ND	21.130±0.143	0.058±0.0005	0.001±0.000
3	0.024±0.0015	0.236±0.0001	ND	28.08±0.156	0.077±0.0011	0.005±0.0008
4	0.015±0.0005	0.147±0.0014	ND	42.02±0.6960	0.098±0.0021	ND
5	0.018±0.0005	0.123±0.056	ND	19.72±0.3250	0.130±0.0011	ND
6	ND	0.142±0.0005	ND	36.78±0.1080	0.072±0.0054	ND
7	ND	ND	ND	1.627±0.8349	0.012±0.0009	ND
8	ND	0.011±0.0029	0.002±0.0024	5.739±2.5246	0.006±0.0099	ND
Av.	0.0264	0.1413	0.002	22.897	0.074	0.0026
**	ND	0.009±0.0028	ND	1.013±0.3219	0.003±0.0043	ND

** Mean the normal woman

Table 4 The comparission of the heavy metals concentration in blood samples with other countries.

Reference	A mount of metals mg/L							
	Pb	Zn	As	Mg	Co	Sn	Fe	Cr
Stasys (2007)	0.77 µg/g	7.47 µg/g	-	-	-	-	-	1.21 µg/g
Blooma(2011)	15.54, 15.44	5.05, 4.93	4.10, 4.33	32.79, 34.52	-	230.43, 223.00	-	-
Iman (2011)	2.897±1.851 µg/dl	-	-	-	-	-	-	-
Fernando(2011)	43.39±52.65 µg/l	-	-	-	-	-	-	1.31±3.01 µg/l
Kazi (2008)		4.00					6.80	5.55
Sekhar(2009)	10.3-16.2 µg/g	2.2-8.5 µg/g	8-35 µg/L	-	1.2-2 µg/g	-	-	8.2 -12.2 µg/g
Salih (2012, this study)	0.0786	1.1332	-	3.840	-	0.01225	23.82	0.0122

Table 5 The comparison of the heavy metals concentration in urine samples with other countries.

Reference	A mount of metals mg/L						
	Pb	Zn	As	Mg	Co	Fe	Cr
Fernando(2011)	22.284±29.92	-	-	-	-	-	0.95±1.89
Sekhar (2009)	20-44	1.1-2.1	11-38 µg/L		NF	-	2.1-6.3
Kazi (2008)	-	2.93				1.69	5.35
Salih(2012, this study)	0.0264	0.1413	0.002	22.897	-	0.074	0.00266

Table 6 The wave length of heavy metals of blood and urine samples

Parameter	A mount of metals mg/L							
	Pb	Zn	As	Mg	Co	Sn	Fe	Cr
Wavelength(nm)	220.353	206.200	193.696	285.213	228.616	189.927	238.204	267.716
Concentration (blood)	0.0786	1.1332	ND	3.8406	ND	0.01225	23.82	0.0025
Concentration (urine)	0.0264	0.1413	0.002	23.428	ND	ND	0.074	0.0026

Table 7 difference among metals in blood

Metals	Number	Blood concentration			
		Mean rank	Chi-squar	df	<i>p</i> value
Pb	8	15.13	40.368	5	< 0.001
Zn	8	23.50			
Mg	8	31.50			
Sn	8	7.88			
Fe	8	39.50			
Cr	3	2.00			
Total	43				

Kruskal-Wallis test

Table 8 difference among metals in urine

Metals	Number	Blood concentration			
		Mean rank	Chi-squar	df	<i>p</i> value
Pb	5	9.20	24.142	4	< 0.001
Zn	7	17.71			
Mg	8	27.50			
Fe	8	12.50			
Cr	3	2.00			
Total	31				

Kruskal-Wallis test

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