Lead (Pb) Exposure From Outdoor Air Pollution: A Potential Risk Factor For Cervical Intraepithelial Neoplasia Related To HPV Genotypes

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Abstract

Human papillomavirus genotypes (HPVs) have been confirmed to be the major cause for Cervical Intraepithelial Neoplasia (CIN) that remains to be one of the most common women cancer around the world. It seems other risk factors connect to the occurrence of cervical cancer include smoking, dietary pattern, sexual behaviour, ethnicity, epigenetics and environmental hazardous materials. Our study characterized the potential cancerous role of Lead (Pb) as a common toxic environmental pollutant agent on CIN outcomes.

The concentration of Pb was quantified using atomic absorption spectrometer in the liquid based cytology specimens of 40 CIN subjects, 50 HPV infected-non cancerous cases and 43 non-HPV infection/non-cancerous women.

The Pb concentration was 5.5 (4.7–6.4) $\mu\text{g/dL}$, 4.7 (4.2–8.7) $\mu\text{g/dL}$ and 4.7 (4.5–5.4) $\mu\text{g/dL}$ in CIN group, control group with HPV infection and non-HPV/non-cancerous group, respectively. The results showed higher Pb concentration is associated with higher risk for cervical malignancy in comparison with non-HPV/non-cancerous subjects, after controlling for age effect (aOR = 4.55, 95% CI: 1.55–15.07, $P < 0.01$).

Our finding suggested a direct significant association between Pb accumulation and CINs existence related to HPV infection. The consequence needs to be further validated by controlling the confounders to better understanding of Pb impact from outdoor air pollution on cervical cancer.

1. Introduction

Cervical cancer is the fourth most common diagnosed cancer among women and causes substantial deaths globally. Although cervical cancer incidence has been declining in recent years for the effective screening and vaccination programs. On the other hand, cervical cancer related to human papillomavirus (HPV) remains to be a severe public health concern in undeveloped countries for a lack of resources and organized monitoring programs. The incidence of cervical cancer varies in undeveloped and developed communities from 11.3 to 18.8 per 100, 000 women in 2020, respectively. However, the incidence of cervical cancer is being increasing in the past decade. It seems several potential risk factors including HPV infection, life style and self-awareness have been associated to cervical malignancies (Momenimovahed & Salehiniya, 2018; Sohrabi & Hajia, 2017; Sung et al., 2021; Vafaeinezhad et al., 2018). Persistent and colonization of high-risk HPV genotypes in the genital tract explains almost all cases in cervical dysplasia or cervical cancer (Hajia & Sohrabi, 2018; Stanley, 2010).

In addition to HPV infection, multiple environmental factors are also shown to be involved in the risk profile of cervical cancer. No surprisingly, factors associated with the acquisition or the pathogenic progress of HPV play an important role for precancerous abnormalities to cervical cancer. Those factors include early age of first intercourse, sexually transmitted infections (STIs), multiple sexual partners, multiple pregnancies, tobacco, smoking, lack of fruits and vegetables in diets (Cohen, Jhingran, Oaknin, & Denny, 2019; Sohrabi, Hajia, Jamali, & Kharazi, 2017). Besides, accumulating evidence is suggesting a

contributing risk effect of heavy metals as well. Heavy metal refers to the metallic elements with relative higher density of greater than 5 g/cm³. Some of the them are essential for biological progress, while they can be deleterious and may cause cancer after exposure to high quantity or long-term exposure to even small quantity(Engwa, Ferdinand, Nwalo, & Unachukwu, 2019). Among a wide range of heavy metals in the environment, Lead (Pb) is a kind of widely distributed toxic environmental pollutant and Pb is classified as possible carcinogenic group 2B to human(Fasinu & Orisakwe, 2013; Humans, 2006).

It can be accumulated and identified from soil waste, drinking water, smoke, air, fruits and vegetables due to air pollution by industrial factories and fuel vehicle (traffic congestion). Scientific literatures have shown a link between Pb and gastrointestinal, lung, bladder and head-neck cancers(Cobanoglu, Demir, Sayir, Duran, & Mergan, 2010; Khlifi & Hamza-Chaffai, 2010; Sadetzki, Bensal, Blumstein, Novikov, & Modan, 2000; Turkdogan, Kilicel, Kara, Tuncer, & Uygan, 2003; Yuan, Yang, & Li, 2016).

However, findings about the association between Pb concentration and cervical cancer are limited. Current studies mainly focus on characterizations of Pb concentration from cervical scrapping specimens of women who live in Tehran, which is the most polluted cities in the world. Hence, we have appraised and assessed the association between Pb concentration in women suffered from CINs related to HPV genotypes and non-HPV/non-cancerous outcomes, using of liquid based cytology specimens.

2. Materials And Methods

2.1. Study Population and Data Collection

A total of 133 liquid based cytology specimens were obtained from Moharreri et al. and Sohrabi et al studies including 40 cases with cervical intraepithelial neoplasia (CIN), 50 HPV infected-non cancerous cases and 43 non-HPV infection/non-cancerous women. Subjects were confirmed by histopathology examinations and HPV genotyping was also identified using home-brew qPCR assay. In order to meet ethical considerations, the study was performed in accordance with the 1964 declaration of Helsinki and its later amendments. Each cancer subject was informed about the objectives of the study and signed a consent form before entering the study. The controls were residual of archival specimens from other studies and necessary clinical data was collected from medical documents. HPV genotyping consequences of the subjects were divided into different categorizes based on the HPV DNA genotypes such as high-risk HPVs 16 and 18, low-risk HPVs 6 and 11, single and multiple high-risk HPV genotypes(Moharreri & Sohrabi, 2021; Sohrabi et al., 2014; Sohrabi, Rahnamaye-Farzami, Mirab-Samiee, Mahdavi, & Babaei, 2016).

2.2. Lead (Pb) Measurement

Pb was measured using an Atomic Absorption Spectrometer (Agilent technologies/200 Series®, USA). The AA Spectrometer equipped with a GTA120 Graphite tube atomizer and auto sampler. Pyrolytic ally coated furnace tubes were employed and trace metal-free polycarbonate tubes were used for sample preparation. GFAAS conditions: 283.3 nm wavelength, 7A slit, D2 background correction, grooved furnace

tube. Dry: ambient to 125°C in 15-second ramp, 5 second hold. Ash: 125°C to 600°C in 45-second ramp, 20 second hold. Atomize: 600°C to 2400°C in fast ramp or step, 5 second hold.

We used the Milli-Q water purified by de-ionization with a Milli-Q system (Millipore) for washing all laboratory ware, solutions and standards preparations. In addition, all reagents were obtained from Merck Co. Working standards were prepared daily by serial dilution of a master standard with 0.1 % v/v nitric acid. A dilution range of Pb standards was made and vortexed by 0, 1, 5, 10, 25, 50 and 100 µg/dL for working standard solutions. Calibration was also be performed directly by using aqueous standards.

The 100 µl samples were diluted with 400µl of “Matrix modifier” (contains 0.1 % v/v nitric acid, 0.2 % m/v ammonium dihydrogen phosphate and 0.5 % m/v Triton X-100 was used throughout). The sample lead concentrations were calculated from the integrated absorbance measurements and the calibration graph. Analysis was performed with 20 µl loads with the following GFAAS conditions.

The method was evaluated and verified for accuracy and precision. The limit of detection, defined as 3 times the standard deviation (SD) of the blank signal that was 0.2 µg/dL, corresponding to a limit of quantification (10× SD) of 0.6 µg/dL. Precision was 6.7% at 10 µg/dL ($n = 20$) and 2.7 at 25 µg/dL ($n = 20$). The recovery of 25 µg/dL was 97.2%. Accuracy was checked by analyzing standard reference materials: Seronorm™ (Trace Elements Serum) an accuracy control for the analysis of trace elements and heavy metals(Md Noh, Ismail, & Surif, 1977; Taupeau, Poupon, Nome, & Lefevre, 2001; World Health & Inter-Organization Programme for the Sound Management of, 2011).

2.3. Statistical analysis

The Pb concentration was first investigated as numerical variable and presented in CIN group, HPV infection no cancerous cases and no-HPV/no-cancerous subjects. Because the Pb concentration was skewed distributed in the study population, it was summarized using median value and corresponding interquartile range in each group and compared using non-parametric methods. Specifically, we used Mann-Whitney *U* test to compare the difference of Pb concentration between case and control groups, and Kruskal-Wallis test to compare the difference between CIN grades. Then, we divided the age into greater than or equal to 35 and below 35 years and compared the Pb concentration between the two age groups in each population study. Furthermore, we used Spearman correlation analysis to estimate the correlation between Pb concentration and age of the subjects in each group.

We further divided the Pb exposure into low concentration and high concentration by its median value among all subjects and categorized it into low, middle and high level groups by its quartile concentrations. Then, we investigated the association between different levels of Pb exposure and CIN using logistic regression model, controlling for age effect and HPV genotypes. CIN cases were compared specially with HPV positive control and HPV negative controls, separately. Results were reported as odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

In addition, we studied the Pb concentration in different HPV groups based on their genotypes. Findings were presented as box plot and summarized using median value and interquartile ranges. The difference

of Pb concentrations was compared between HPV infected and non HPV infected women by the Mann-Whitney U test, and the comparison of Pb concentration between multiple HPV genotypes was performed using Kruskal-Wallis test. The statistical analysis was conducted using R software (version 3.6.1), and a two-tailed P value ≤ 0.05 was regarded to be statistical significant.

3. Results

3.1. Pb Consequences

Pb concentration was measured on 133 subjects and the median concentration of Pb was higher in the cancer group comparing to the total control group ($P < 0.01$). As a result, when control group was further divided into HPV infected and no- HPV infected, the Pb level in the case group was only statistically higher than the no- HPV infected women ($P < 0.01$). The median Pb concentration was also different within CIN grades ($P = 0.019$). The outcomes are presented in Table 1.

Table 1
Characteristics of Lead (Pb) Concentration in CIN Subjects and Controls (n = 133).

Research group	Pb Concentration (µg/dL)	
	Median	Interquartile Range
CIN (N = 40)	5.5	4.7–6.4
CIN I (N = 6)	6.6	6.4–7.0
CIN II (N = 6)	5.0	4.7–5.8
CIN III (N = 28)	5.4	4.7–6.2
Control (N = 93)	4.7	4.3–5.8
No-Cancerous- HPV Infection (N = 50)	4.7	4.2–8.7
No-Cancerous- no HPV Infection (N = 43)	4.7	4.5–5.4
Comparisons	P value	
CIN versus Overall Controls	< 0.01 ^a	
CIN versus HPV Infected Control	0.080 ^a	
CIN versus no-HPV Infected Control	< 0.01 ^a	
Comparison between CIN Grades	0.019 ^b	
Abbreviations: CIN, Cervical Intraepithelial Neoplasia; HPV, Human papillomavirus; Pb, lead.		
^a <i>P</i> value in the comparison between two indicated groups has been obtained by Mann-Whitney <i>U</i> test.		
^b <i>P</i> value in the comparison between three groups has been obtained by Kruskal-Wallis test.		

3.2. Pb Concentration and Age

The difference of Pb concentrations was also examined in two age groups and its potential linear correlation with age as a numerical variable, in each study group. Outcomes are shown in Table 2. In the total control group, Pb level was higher in age below 35, compared to age greater than 35, however without linear correlation ($P_{\text{difference}} = 0.04$). In the non-HPV infection/non-cancerous group, the Pb concentration was higher in the younger age group and was negative linearly correlated with age ($P_{\text{difference}} = 0.01$; $r = -0.29$, $P_{\text{correlation}} < 0.01$), while such pattern was not observed in the HPV infected-non cancerous cases.

Table 2
Pb Concentrations in Different Age Groups within Population Study and its Linear Correlation.

Population Study	Pb concentration		p^a difference	r	p^b correlation
	Median	Interquartile range			
CINs					
< 35 (N = 6)	5.1	4.7–6.4	0.58	0.00	0.972
≥ 35 (N = 34)	5.6	4.7–6.4			
Controls					
< 35 (N = 60)	5.0	4.5–6.3	0.04	-0.13	0.072
≥ 35 (N = 33)	4.6	4.3–4.8			
No-Cancerous- HPV Infection					
< 35 (N = 36)	4.8	4.2–10.1	0.69	-0.01	0.927
≥ 35 (N = 14)	4.6	4.3–4.9			
No-Cancerous- no HPV Infection					
< 35 (N = 24)	5.1	4.6–5.8	0.01	-0.29	< 0.01
≥ 35 (N = 19)	4.6	4.3–4.7			
Abbreviations: CIN, Cervical Intraepithelial Neoplasia; HPV, Human papillomavirus; Pb, lead.					
^a Compare with the differences in the Pb concentration between two age groups, Mann-Whitney U test.					
^b P value for the spearman correlation between Pb concentration and age.					

3.3 Association between Pb level and CINs

The association between binary Pb level and CINs, in comparison with total control group, control group of HPV infected-non cancerous subjects and non-HPV infection/non-cancerous subjects, is shown in Table 3, separately.

Table 3

Risk Analysis for CIN in Association with Higher Level of Pb Concentration, which is Categorized by its Median Level in all Subjects, Comparing with Different Control Groups.

N (Case/Control)		OR ^a (95% CI)	<i>P</i>	aOR ^b (95% CI)	<i>P</i>
Compare with overall controls					
Low (< 4.8)	11/50	1.00 (Ref.)	< 0.01	1.00 (Ref.)	< 0.01
High (≥ 4.8)	29/43	3.07 (1.40–7.09)		3.61 (1.43–9.87)	
Compare with no-Cancerous- HPV Infection					
Low (< 4.8)	11/26	1.00 (Ref.)	0.021	1.00 (Ref.)	0.104
High (≥ 4.8)	29/24	2.86 (1.20–7.14)		2.43 (0.85–7.36)	
Compare with no-Cancerous- no HPV Infection					
Low (< 4.8)	11/24	1.00 (Ref.)	0.010	1.00 (Ref.)	< 0.01
High (≥ 4.8)	29/19	3.33 (1.35–8.59)		4.55 (1.55–15.07)	
Abbreviations: CIN, Cervical Intraepithelial Neoplasia; HPV, Human papillomavirus; Pb, lead; OR, odds ratio; aOR, adjusted odds ratio; 95% CI, 95% confidence interval.					
^a Unadjusted OR estimated by logistic regression model.					
^b Adjusted OR estimated by logistic regression model, controlling for women’s age effect in the model.					

The Pb exposure was categorized by the median concentration (4.8 µg/dL) in all the women included in the study. After controlling for age, higher Pb level was associated with a higher risk of CIN in comparison with total control group (aOR = 3.61, 95% CI: 1.43–9.87, *P* < 0.01), as well as in the comparison with control group without HPV infection (aOR = 4.55, 95% CI: 1.55–15.07, *P* < 0.01). No statistical significance was between Pb and CIN in comparison with control group infected of HPV.

The association between ternary Pb level and CIN (low: < 4.6, middle: 4.6–5.7, high: > 5.7 µg/dL) was conducted in comparison with different control groups. As shown in Table 4, after controlling for age effect in the regression model, higher level of Pb concentration was associated with a higher risk for CIN in the comparison with total control group (aOR = 5.72, 95% CI: 1.87–19.73, *P* < 0.01). Moreover, greater OR was observed when comparing to non-HPV infection/non-cancerous controls (aOR = 7.01, 95% CI: 1.89–29.91, *P* < 0.01).

Table 4

Risk Analysis for CIN in Association with Higher Level of Pb Concentration, which is Categorized by its Tertile Value in All Subjects, Comparing with Different Control Groups (n = 133).

	N (Case/Control)	OR ^a (95% CI)	P	aOR ^b (95% CI)	P
Compare with overall controls					
Low (< 4.6)	8/41	1.00 (Ref.)		1.00 (Ref.)	
Middle (4.6–5.7)	13/27	2.47 (0.92–7.00)	0.078	2.34 (0.72–8.07)	0.162
High (> 5.7)	19/25	3.90 (1.53–10.70)	< 0.01	5.72 (1.87–19.73)	< 0.01
Compare with no-Cancerous- HPV Infection					
Low (< 4.6)	8/22	1.00 (Ref.)		1.00 (Ref.)	
Middle (4.6–5.7)	13/12	2.98 (0.98–9.55)	0.058	2.26 (0.57–9.43)	0.250
High (> 5.7)	19/16	3.27 (1.12–9.70)	0.027	3.40 (0.99–12.94)	0.059
Compare with no-Cancerous- no HPV Infection					
Low (< 4.6)	8/19	1.00 (Ref.)		1.00 (Ref.)	
Middle (4.6–5.7)	13/15	2.06 (0.69–6.46)	0.203	2.01 (0.54–7.95)	0.305
High (> 5.7)	19/9	5.01 (1.65–16.58)	< 0.01	7.01 (1.89–29.91)	< 0.01
Abbreviations: CIN, Cervical Intraepithelial Neoplasia; HPV, Human papillomavirus; Pb, lead; OR, odds ratio; aOR, adjusted odds ratio; 95% CI, 95% confidence interval.					
^a Unadjusted OR estimated by logistic regression model.					
^b Adjusted OR estimated by logistic regression model, controlling for women's age effect.					
Tables 5. Women Risk Analysis for CINs Compared with HPV Genotypes.					

3.4. Pb Concentration and HPV Genotypes

Pb concentrations in different HPV genotypes groups are shown in Fig. 1. Moreover, the difference of Pb concentration was compared between women with and without HPV infection among all subjects or restricted in control group; and the difference of Pb concentration within groups with different HPV genotypes, but there was not any significant differences between groups (results are not shown). HPV genotype was also included as a confounding variable in the model comparing cases with controls infected with HPV to assess the association between Pb level and outcome, but the ORs were not substantially altered (Table 5).

Tables 5. Women Risk Analysis for CINs Compared with HPV Genotypes.

	N (Case/Control)	aOR (95% CI) ^a	P
Pb Concentration Categorized by its Median Value			
Low (< 4.8)	11/26	1.00 (Ref.)	
High (≥ 4.8)	29/24	1.33 (0.35 - 5.03)	0.673
Pb Concentration Categorized by its Tertile Values			
Low (< 4.6)	8/22	1.00 (Ref.)	
Middle (4.6-5.7)	13/12	2.04 (0.40 - 10.95)	0.392
High (> 5.7)	19/16	3.13 (0.61 - 18.38)	0.180

Abbreviations: CIN, Cervical Intraepithelial Neoplasia; HPV, Human papillomavirus; Pb, lead; aOR, adjusted odds ratio; 95% CI, 95% confidence interval.

^a Adjusted OR estimated by logistic regression model, controlling for age and HPV genotypes.

4. Discussion

Tehran, a metropolitan city with a population of more than 10 million, is one the most pollutant area throughout the world. Air pollution is a life-threatening factor caused by urbanization and industrialization, especially due to vehicle fuel. Therefore, it seems traffic congestion is a common source of Pb emission to the environment (Ali asghar, 2021; Kermani, Dowlati, Jonidi jafari, & Rezaei Kalantary, 2016; Khorrami et al., 2021).

Pb is used widely in the industry and domestic settings, however, women could possibly more exposed to Pb via ambient environment, cooking and cosmetics. Lead (Pb) is studied to be toxic for multiple organs and may contribute to malignancies at even a low dose after a long-term exposure. Many studies have confirmed a link between Pb exposure and human cancerous alterations in liver, kidney and brain for their sensitivity to Pb toxicity. Specifically, Smoking women have a higher Pb concentration in the endo-cervical tissues than non-smoking women. However, there is not a significant association between Pb and cervical cancer and the Pb effect on women malignancies is unknown. Elevated blood Pb level is known to be detrimental on reproductive health, birth outcomes and hormonal functions. Pb potential carcinogenic mechanism is not fully understood yet. However, some evidences demonstrated an excess Pb replaces zinc in some regulatory proteins, thus it can be accumulated in blood cell and therefore be transported to other organs in animal model. Pb could cause direct DNA damage by inducing free radicals that causes oxidative stress damage for DNA and chromosomal. Notably, its tumorigenesis effect lies in its supportive ability to impair DNA synthesis and repair system. Therefore, the synergetic effect between Pb and other carcinogens, particularly Cadmium, for the origination of tumours in cervix, may be considered in further studies (Caffo et al., 2014; Fenga, Gangemi, Di Salvatore, Falzone, & Libra,

2017; Kumar, 2018; Marouf, 2018; Matovic, Buha, Ethukic-Cosic, & Bulat, 2015; Rzymiski et al., 2016; Sanders et al., 2015; Silbergeld, 2003; Silbergeld, Waalkes, & Rice, 2000; Wilk et al., 2017).

The microbial pathogens might also play a synergistic role in the progression of cervical cancer in the existence of carcinogenic heavy metals and trace elements. The scientific literatures suggested Pb is associated with change of miRNA expression in cervix through epigenetic regulations, immunotoxin effects by dysregulating cytokine productions, promoting inflammation, and altering the expression and activity of T helper cells (Fenga et al., 2017; Rzymiski et al., 2016). Nevertheless, none of the proposed mechanism could fully explain the carcinogenic role of Pb or its differential expressions in multiple organs, further studies are still needed. In line with our results, a previous study also reported a higher Pb level in CIN endocervical tissues, compared to histological normal tissues. Notably, their study had 3 CIN cases, while our study focused on bigger sample size, ensuring a better statistical power (Rzymiski et al., 2016; Sanders et al., 2015).

Our study adds up to the direct evidence that higher Pb level accumulated in the cervical tissue is associated with higher risk of cancerous changes. There are also significant drawbacks in our study. Firstly, our study could not exclude the effects of factors that may affect Pb exposure such as smoking habits, education, living conditions and working environment. Albeit, smoking habit is not common in Iranian women. We considered the education, smoking habits and environment variables in the questionnaire, but they have not been filled in that, so the insufficient factors excluded for biostatistical analysis. Secondly, for the cross-sectional setting of the study design, we could not elucidate the possible causal relationship between Pb exposure and cervical cancer. It could be Pb triggered cancerous changes in the organ, but it can also be the cancerous changes in the cells increased the affinity between Pb and cervical tissues.

5. Conclusion

Here, the study showed an association between increased cervical Pb concentration and cervical intraepithelial neoplasia changes in comparison with non-HPV/non-cancerous subjects, after controlling for age effect. However, the higher level of Pb concentration in cervical tissue is correlated with CIN grades 2 and 3 in comparison with no-HPV infection subjects. Therefore, further dedicated studies are needed to appraise the source of Pb exposures particularly from outdoor air pollution and its consequences on cervical cancer progression.

Declarations

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Figures

Figure 1

Boxplots of Pb concentration in each group based on HPV genotypes. In the boxplot of each group, the median concentration is the vertical bold line inside the box, and the 25th and 75th percentiles are the left and right vertical bounds of the box, respectively. The horizontal line represented the range between 1.5 times interquartile below 25th percentile and 1.5 times interquartile above 75% percentile. In addition, the points were measurements below or above 1.5 time's interquartile range.