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Relationships of the serum levels of toxic trace elements in pregnant women versus exposure to second-hand smoke

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Tobacco smoke contains many toxic heavy metals that cause adverse effects in humans. The association between second-hand smoke (SHS) and the toxic metals accumulated in pregnant women are still unclear. We measured trace element levels in the sera of pregnant women exposed to SHS and compared the data to those of unexposed pregnant women. Moreover, the data were contrasted with the anthropometric measurements (birth weight, birth length, and head circumference) of their newborn babies after delivery. Two groups of pregnant women were voluntarily recruited, and their blood samples were collected. Then, ten trace elements were measured in their sera, and the data were statistically analyzed using R version 4.0.3 software. The serum trace elements in the smoking subjects were higher than those of the non-smokers, but the difference was not statistically significant ($p > 0.05$). The SHS had adverse effects on some trace elements on the smokers' sera. The concentrations of Cr and Ni in mothers exposed to SHS (32.85–51.25) were significantly higher than those in the mothers unexposed to SHS (28.26–44.80; $p < 0.05$). The study found that some trace elements significantly affected the anthropometric measurements of infants born to mothers who were exposed to SHS ($p < 0.05$). Exposure of pregnant women to cigarette smoke had adverse effects on their newborns' body weights. The mothers who smoked had babies with lower weights. Also, the exposure to cigarette smoke might have caused some of the disorders during their pregnancy.

Keywords Cigarette smoking, Trace elements, Newborn, Pregnant women, Tobacco smoke pollution

Nearly 20% of the world's population smoke tobacco products, and the global statistics predict that the number of smokers may rise to 1.6 billion by the end of 2025[1]. In 2019, tobacco smoking was associated with 8.71 million deaths worldwide, comprising 13.6% of all human deaths[2]. Tobacco smoke contains approximately 7,000 chemicals and elements, at least 69 of which are known to be carcinogenic. Also, smoking cigarettes is responsible for about 30% of all deaths due to cancer of various bodily organs worldwide[2,3].

Consuming tobacco products and smoking cigarettes during pregnancy are the major modifiable risk factors associated with adverse effects on the babies born to smoking mothers. Exposure to tobacco smoke, also referred to as second-hand smoke (SHS), can be due to direct exposure to people who smoke cigarettes. This is responsible for approximately 1% of all cases of human deaths worldwide. Second-hand smoke usually lingers indoors for hours and may become more toxic over time, depending on the frequency and quality of certain environmental variables. These include airflow patterns, ventilation, and physical distance between nonsmokers and smokers[4,5], which can affect the intensity and duration of exposure to the smoke. Exposure to SHS can also

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occur when non-smokers inhale the smoke blown out by smokers at close distance, or if tobacco products are being burnt on such devices as hookahs. In this context, homes and work places are the main areas where people may be exposed to SHS[6].

Health disorders associated with cigarette smoking are attributed to the inhalation of various toxic substances present in the smoke, including nitrosamines, polycyclic aromatic hydrocarbons, volatile organic compounds, and other chemicals[7]. Arsenic (As), cadmium (Cd), and lead (Pb) are among the most common trace elements linked to adverse health effects in cigarette smokers[8]. These are known to be carcinogenic in humans by the International Agency for Research on Cancer[9]. Trace heavy metals with relative high densities can have adverse effects on the tissues of human, animals and plants[10]. Although some heavy metals, such as iron (Fe), zinc (Zn), and copper (Cu) are essential for human metabolic activities, their concentration can become harmful to human health when levels exceed a certain threshold[11]. The resultant health issues may include neurological, cardiovascular, renal injuries, and endocrine disorders, in addition to cancers and infertility risks[12].

Accumulation of trace elements in the reproductive tissues of men and women can lead to the infertility[13]. Also, trace elements, such as As, Cd, and Pb, are associated with increased oxidative stress, cell apoptosis, endocrine disruption, and epigenetic damages[14]. Inhalation of SHS during pregnancy increases the risk of premature birth, and low birth weight, and is associated with a high incidence of maternal anemia[16,16]. Limited studies have been conducted on the adverse effects of SHS in pregnant women. In this context, the impact of SHS in the Iranian pregnant women has not been studied. Therefore, this study was planned to investigate the adverse effects of SHS on the pregnant women residing in Kermanshah province, Iran.

Materials and methods

Study population

This study was designed as a case-control research project aimed at investigating the effects of SHS on pregnant women versus a similar but unexposed population. The study protocols including the consent form were reviewed and approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (approval code: IR.KUMS.REC.1402.510).

Determination of sample size

The required sample size of 111 subjects was determined based on the following formulas 1 and 2, and our experience from a former study[17]. See reference 1 in the footnote below.

$$n_1 = \frac{(r+1)}{r} \times \frac{\sigma^2 (Z_{1-\beta} + Z_{\alpha/2})^2}{(d)^2} \tag{1}$$

Where $\boldsymbol{n}_1$ is the sample size in the smaller group, $\boldsymbol{r}$ is the ratio of the size of the larger group to the smaller group, $\boldsymbol{\sigma}$ is the standard deviation of the considered variable, and $\boldsymbol{d}$ is the smallest difference between the means of the two groups that is considered significant. $1-\boldsymbol{\beta}$ and $\boldsymbol{\alpha}$ are the values corresponding to the power and type I error, respectively. The parameter $\boldsymbol{d}/\boldsymbol{\sigma}$ is the effect size. For roughly equivalent selection of both groups ($r \cong 1$), $\boldsymbol{\alpha} = 0.05$, $1-\boldsymbol{\beta} = 0.8$ and an effect size of 0.6, the minimum required sample size is 44 subject in each group.

$$n_1 = \frac{(1+1)}{1} \times \frac{(0.84+1.96)^2}{(0.6)^2} \cong 44 \tag{2}$$

Taking into account a possible dropout rate of about 13%, in the present study we included 57 mothers for the smaller group.

Subject's recruitment & grouping

The pregnant women referred to a local obstetric ward were notified of the impending research project, and they were invited to join the study as subjects. From among the pregnant women who indicated their interest in joining the study, we began recruiting the eligible ones until we reached 111 subjects, based on the inclusion and exclusion criteria. After attending an orientation session and personal interviews, these women reviewed the study protocol and signed an informed consent letter before being officially included in the study. These otherwise healthy subjects were divided into two groups of exposed versus non-exposed pregnant women to SHS. They were entered in a checklist anonymously by codes under demographic classification as age, level of education, employment status, number of previous births, smoking status, history of abortion, and BMI.

Data collection

At each experimental session two of the investigators were assigned to interview the subjects for that session, collect their demographic data in a checklist, and draw the 3-ml venous blood samples after interview and grouping of the women at Motazedi and Imam Reza Hospitals, in Kermanshah, Iran. Also, after delivery of their babies, the physical features of each newborn (birth weight, birth length, and head circumference) was recorded in an approved checklist by two attending physicians specialized in obstetrics and gynecology that were fully familiar with the women.

Inclusion and exclusion criteria

The inclusion criteria required that each pregnant woman had lived in Kermanshah region for at least five years. They had the choice of being exposed to SHS or not at all at home, or work place or both. Also, an attempt was made to select pregnant smokers and non-smokers from a geographic area with a similar lifestyle. Pregnant women with diabetes, chronic diseases or exposure to industrial sites, and those who had lived in that region for less than 5-years were excluded from the study. The demographic variables of these women were recorded in a checklist. Finally, the birth weight, length, and head circumference of each newborn baby were carefully taken and recorded shortly after birth.

Processing sera for trace elements

Before delivery, a venous blood sample (2-ml) was collected from each of the women, and was centrifuged at 3000 rpm for 10 min. Next, the serum samples were transferred to micro-centrifuge tubes, labeled with each patient's code and stored in a freezer at -20 °C until further processes. The serum samples (1-ml each) were mixed with 3-ml nitric acid (Suprapure HNO3, 65%, Merck, Germany) and 1-ml hydrogen peroxide (H_2O_2; 30%), and were incubated in a Bain-Marie water bath at 98 °C[17] for about six hours. Briefly, this process was performed in three steps as outlined below: Step 1: 3-ml nitric acid was added to each of the serum samples and kept at room temperature for 24 h, allowing for a slow digestion process. Step 2: 1-ml hydrogen peroxide was added to the samples and incubated in a Bane Marie water bath (TW12, Julabo, Germany) until the digestion process was complete. The clear solution point became cloudy after six hours, which was brought to a volume of 10-ml by adding distilled water. Step 3: The samples were then injected into an inductively coupled plasma mass spectrophotometer (ICP-MS; Perkin Elmer-7300 DV; Florida, USA) for the determination of the trace elements in the serum samples.

Statistical analyses

For quantitative variables, we first checked the data for normal distribution using the Shapiro-Wilks' test. Then, we used the data means and standard deviations to analyze the variables. If the date were not normally distributed, we used the median and interquartile ranges. For qualitative variables, we reported the frequency numbers and percentages. To examine the relationship between quantitative variables within binary variable levels, we used Student's *t*-test and Mann-Whitney's U test. For multi-class qualitative variables, we used the analysis of variance (ANOVA) and Kruskal-Wallis tests. Next, we used Pearson's and Spearman's correlation coefficients, respectively, for normally and non-normally distributed data. These were presented using a heat plot format. Multivariate regression analysis was used to examine the simultaneous effect of trace metal concentrations versus the weight, height and head circumference of the babies. These data were separated into two groups: those exposed or not exposed to SHS. Also, multiple regressions were performed to examine the effect of SHS exposure versus trace element concentrations in the presence of other variables. The statistical significance for all tests was set at $p \leq 0.05$. All statistical analyses were performed, using R software, version 4.0.3 (Posit, PBC; Vienna, Austria).

Results

Descriptive statistics and univariate analyses

The data, as presented in Table 1, indicated that out of 111 pregnant women who were included in the study, 61.26% were exposed to SHS. The clinical and demographic characteristics of the pregnant women, and their babies versus those unexposed to SHS are reflected in Tables 1 and 2. Based on the statistical normality test, the data for age, body mass index (BMI), newborn babies' weight, length, and head circumference were normally distributed ($p > 0.05$). The women's mean age who were exposed to SHS (32.250 ± 5.42) was significantly higher than those who were not exposed to SHS (28.163 ± 6.82 y) ($p < 0.001$). In addition, the mean weight of the babies whose mothers were exposed to SHS (3351.47 ± 495.20 g) was significantly higher than those of the babies whose mothers were not exposed to SHS (3161.63 ± 463.15 g) ($P = 0.046$).

Most mothers who were exposed to SHS had university education (41.2%), while most unexposed mothers had high school education (55.8%). The difference between the two groups based on education was statistically significant ($P = 0.028$). A large percentage of mothers who were not exposed to SHS (88.4%) had normal delivery and only 11.6% of them required cesarean section ($p < 0.001$). Also, only 7% of mothers unexposed to SHS smoked, while 50% of those who were exposed to SHS were also smokers. The difference between the two groups was statistically significant ($p < 0.001$). As reflected in Table 3, the concentrations of Cr and Ni in the mothers exposed to SHS were significantly higher than those of the unexposed mothers (Cr: 32.85 ± 14.57 Vs Ni: 51.25 ± 22.27; or Cr: 28.26 ± 9.27 Vs Ni: 44.80 ± 12.10). The differences in the serum levels of other trace elements between the two groups were not statistically significant ($p > 0.05$).

Quantitative features	Non-Expose to SHS ($n = 43$)	Expose to SHS ($n = 68$)	Total ($n = 111$)	*p*-value
Age (year)	28.163 ± 6.82	32.250 ± 5.42	30.67 ± 6.30	< 0.001*
BMI (kg/m^2)	29.70 ± 4.03	30.70 ± 4.38	30.31 ± 4.26	0.228
Birth weight (gr)	3161.63 ± 463.15	3351.47 ± 495.20	3277.93 ± 489.79	0.046*
Birth length (cm)	50.67 ± 3.52	51.73 ± 3.26	51.32 ± 3.39	0.109
Head circumference (cm)	32.46 ± 3.31	31.97 ± 2.82	32.16 ± 3.01	0.403

Table 1. Quantitative features of pregnant mothers and their infants according to the state of expose / non-expose to SHS. *Significant test in level 0.05.

Qualitative features		Non-expose to SHS ($n=43$)	Expose to SHS ($n=68$)	Total ($n=111$)	p-value
Employment status	Employee	12(27.9)	27(39.7)	39(35.1)	0.205
	Home-keeper	31(72.1)	41(60.3)	72(64.9)	
Level of education	Illiterate	5(11.6)	4(5.9)	9(8.1)	0.028*
	Primary school	7(16.3)	15(22.1)	22(19.8)	
	High school& diploma	24(55.8)	21(30.8)	45(40.5)	
	College	7(16.3)	28(41.2)	35(31.5)	
Type of delivery	C-Section	5(11.6)	33(48.5)	38(34.2)	< 0.001*
	Natural	38(88.4)	35(51.5)	73(65.8)	
Number of previous births	<=2	36(83.7)	48(70.6)	84(75.7)	0.116
	> 2	7(16.3)	20(29.4)	27(24.3)	
Infant sex	Boy	16(37.2)	37(54.4)	53(47.7)	0.077
	Girl	27(62.8)	31(45.6)	58(52.3)	
Smoking status	No	40(93.0)	34(50.0)	74(66.7)	< 0.001*
	Yes	3(7.0)	34(50.0)	37(33.3)	
History of abortion	No	33(76.7)	42(61.8)	75(67.6)	0.101
	Yes	10(23.3)	26(38.2)	36(32.4)	

Table 2. Qualitative features of pregnant mothers and their infants according to the state of expose / non-expose to SHS. *Significant test in level 0.05.

Heavy metals	Non-expose to SHS	Expose to SHS	Total	p-value
Se (μg/l)	61.26 ± 33.95	75.62 ± 53.56	70.25 ± 46.30	0.131
Zn (μg/l)	363.00 ± 698.38	352.90 ± 577.38	363.00 ± 606.98	0.553
Cu (μg/l)	92.30 ± 65.10	106.250 ± 74.25	100.90 ± 71.70	0.196
Fe (mcg/dL)	96.10 ± 85.90	72.20 ± 107.33	78.20 ± 98.70	0.311
As (μg/l)	5.43 ± 1.32	5.21 ± 1.10	5.34 ± 1.07	0.298
Cd (μg/l)	0.471 ± 0.55	0.634 ± 0.52	0.603 ± 0.52	0.233
Cr (μg/l)	28.26 ± 9.27	32.85 ± 14.57	31.26 ± 11.73	0.006*
Hg (μg/l)	0.347 ± 0.46	0.514 ± 0.46	0.461 ± 0.51	0.108
Ni (μg/l)	44.80 ± 12.10	51.25 ± 22.27	47.90 ± 17.00	0.005*
Pb (μg/l)	4.70 ± 7.30	6.75 ± 9.37	6.30 ± 8.80	0.213

Table 3. Concentration levels (median ± IQR) of heavy metals by expose / non-expose to SHS. *Significant test in level 0.05.

Correlation analyses

Based on the correlation coefficients of the quantitative variables for the mothers exposed to SHS (Fig. 1), the correlation coefficient between Fe and the baby's head circumference (r= -0.252; P-value = 0.038), between Cu and the baby's weight (r=-0.393, P-value = 0.001) and between Cu and the baby's head circumference (r=-0.342; P = 0.004) were weak but significant. Conversely, there was no significant correlation between the demographic and clinical characteristics of the same group of mothers ($p > 0.05$). With respect to the qualitative variables for the mothers unexposed to SHS (Fig. 2), there was a weak but significant correlation only between the serum level of Cu in mothers and the baby's head circumference (r= -0.330; P = 0.031). In these mothers, there was a significant correlation between abortion history and their babies' length (P = 0.028). The mean length of the babies from these mothers (52.80 ± 5.39 cm) was higher than that of the babies of mothers with no abortion history (50.030 ± 2.506 cm). There was no significant relationship among other babies' variables, such as weight, length, and the head circumference in either group of mothers. The median ± IQR values of trace elements for the exposed and unexposed mothers to SHS are presented in Table 4. Based on these results, the serum Hg levels (0.7 ± 0.5) was higher in mothers exposed to SHS aged older than 35 years, than those aged 35 years old or younger (0.4 ± 0.5; P = 0.04).

Regression analyses

Multivariate regression analyses were used to examine the factors that influenced the babies' birth weight, length and head circumference (Table 5). These data were adjusted for other significant variables, such as age, education level and delivery type). Based on the coefficient and P values shown in Table 5, the mean serum Cu level for mothers exposed to SHS had a mildly negative effect on the babies' weight (B = -0.002; P = 0.016). However, the mean Cr level had a significantly positive effect on the babies' weight (B = 0.063; P = 0.044), height (B = 0.329; P = 0.001) and head circumference (B = 0.253; P = 0.005). In the same mothers, the exposure to Cd (B = 1.548;

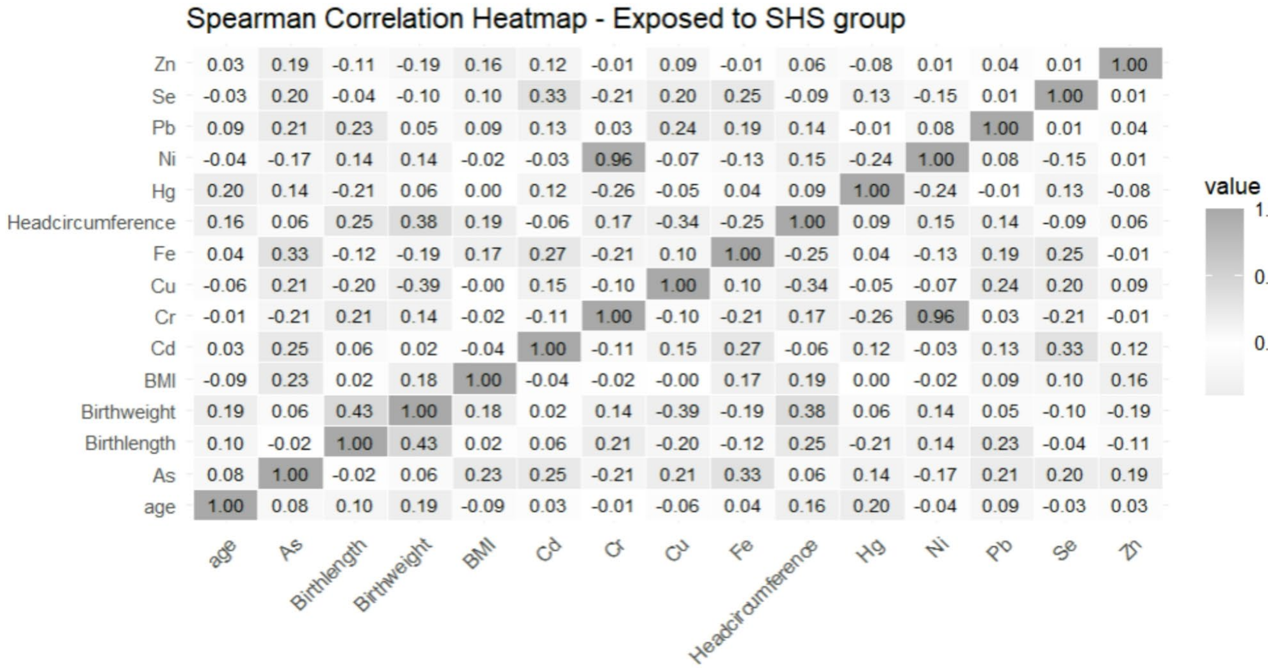

Fig. 1. Heatmap plot related to the Spearman correlation coefficient of quantitative variables investigated in the group of mothers who were exposed to SHS.

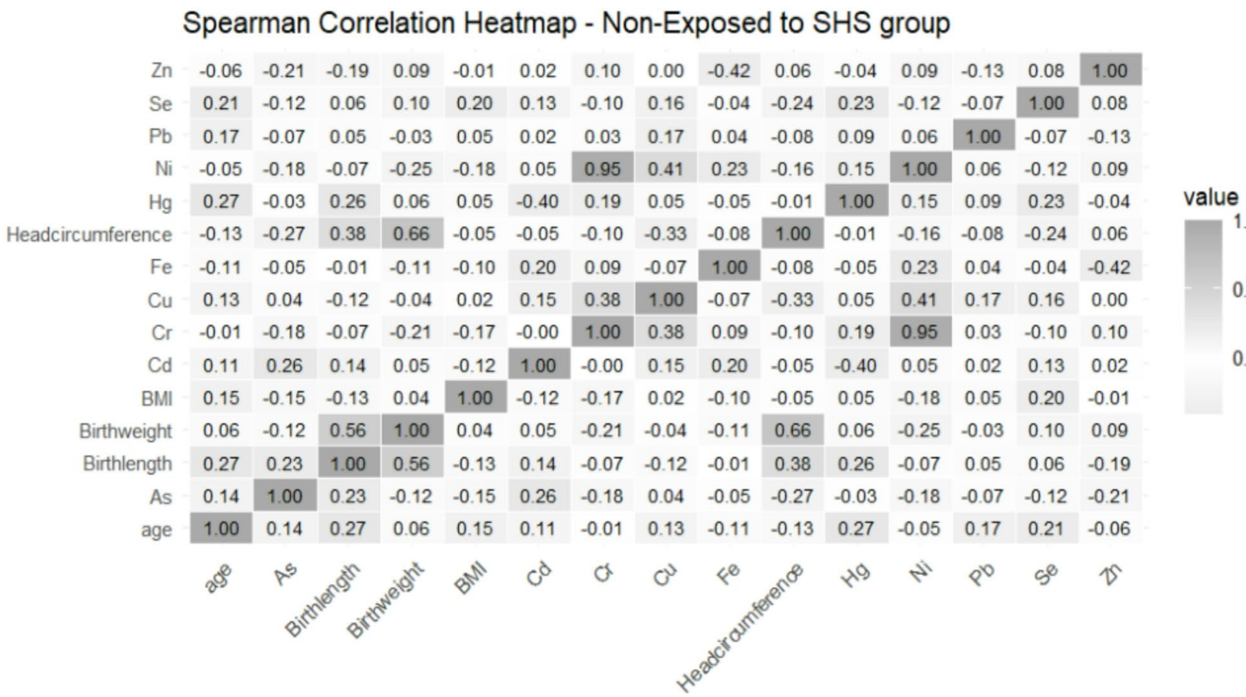

Fig. 2. Heatmap plot related to the Spearman correlation coefficient of quantitative variables investigated in the group of mothers who were not exposed to SHS.

$P = 0.041$) and Pb ($B = 0.112$; $P = 0.033$) correlated with increases in the babies' length. Conversely, increases in the serum levels of Zn,

Hg and Ni correlated with decreases in the babies' length ($B = -0.002$, $P = 0.040$; $B = -1.864$, $P = 0.017$; & $B = 0.234$, $P = 0.002$), respectively. Also, in mothers exposed to SHS, increases in the serum Ni correlated significantly with decreases in the babies' head circumference ($B = -0.140$, $P = 0.036$). In mothers unexposed to

Features		Se	Zn	Cu	Fe	As	Cd	Cr	Hg	Ni	Pb
Age	<= 35	59.8 ± 33.4 78.6 ± 54.4	355.6 ± 709.4 367.2 ± 707.0	91.8 ± 61.7 103.3 ± 89.0	96.4 ± 85.1 65.3 ± 112.5	5.4 ± 1.2 5.1 ± 1.0	0.4 ± 0.5 0.6 ± 0.5	28.6 ± 11.4 32.8 ± 15.5	0.3 ± 0.6 0.4 ± 0.5	44.8 ± 14.8 51.1 ± 25.8	4.5 ± 7.0 6.8 ± 8.6
	> 35	75.4 ± 35.0 68.8 ± 42.6	659.0 ± 494.0 255.0 ± 532.9	114.9 ± 177 118.9 ± 58.6	96.1 ± 121.0 92.6 ± 91.5	5.8 ± 6.1 5.2 ± 1.6	0.6 ± 0.6 0.6 ± 0.5	27.2 ± 8.9 33.3 ± 9.4	0.5 ± 0.3 0.7 ± 0.5	44.3 ± 8.6 52.4 ± 24.3	6.3 ± 11.9 6.4 ± 13.3
	p-value	0.29 0.36	0.68 0.42	0.30 0.97	0.91 0.21	0.48 0.59	0.12 0.62	0.88 0.33	0.52 **0.04***	0.73 0.22	0.50 0.62
Type of Delivery	C-Section	85.0 ± 40.5 78.6 ± 60.7	659.0 ± 794.0 281.7 ± 584.7	101.3 ± 325 117.3 ± 84.2	95.3 ± 67.1 65.3 ± 129.5	5.7 ± 5.8 5.1 ± 0.8	0.4 ± 0.3 0.6 ± 0.4	33.2 ± 15.3 33.1 ± 16.1	0.6 ± 1.2 0.5 ± 0.6	50.6 ± 25.4 51.4 ± 22.0	8.8 ± 9.9 4.4 ± 8.6
	Natural	59.8 ± 26.4 72.1 ± 47.7	360.0 ± 694.7 419.2 ± 555.1	91.8 ± 63.8 100.9 ± 60.3	96.8 ± 114.3 77.8 ± 92.7	5.4 ± 1.1 5.5 ± 4.1	0.4 ± 0.6 0.5 ± 0.5	27.6 ± 9.4 32.2 ± 13.0	0.3 ± 0.4 0.5 ± 0.4	44.7 ± 11.9 49.9 ± 21.3	4.3 ± 8.1 8.6 ± 9.7
	p-value	0.34 0.45	0.44 0.41	0.36 0.94	0.75 0.93	0.29 0.07	0.89 0.22	0.34 0.25	0.19 0.97	0.49 0.27	0.21 0.07
Level of education	Illiterate	61.2 ± 22.0 156.7 ± 141	357.0 ± 538.9 409.4 ± 466.1	65.2 ± 29.8 125.3 ± 15.3	95.3 ± 163.7 175.6 ± 274	5.8 ± 6.9 5.8 ± 3.0	0.7 ± 0.6 0.7 ± 2.0	18.9 ± 10.8 27.5 ± 16.9	0.2 ± 0.5 0.7 ± 0.5	27.9 ± 18.2 46.3 ± 27.8	8.6 ± 9.02 6.0 ± 14.0
	Primary school	65.9 ± 46.7 86.3 ± 57.1	890.3 ± 918.0 200.6 ± 280.8	98.9 ± 58.8 114.2 ± 91.9	96.1 ± 100.5 81.5 ± 58.4	5.4 ± 1.4 5.4 ± 4.1	0.4 ± 0.5 0.5 ± 0.5	36.2 ± 14.0 31.8 ± 8.9	0.2 ± 0.3 0.7 ± 0.5	54.9 ± 18.7 50.5 ± 24.5	4.2 ± 4.2 8.9 ± 14.5
	High school	59.6 ± 76.7 75.2 ± 42.5	322.5 ± 818.1 281.7 ± 658.3	92.3 ± 68.9 129.2 ± 68.2	108.6 ± 142 79.2 ± 108.8	5.2 ± 0.82 5.6 ± 7.9	0.2 ± 0.5 0.6 ± 0.3	24.7 ± 12.7 33.9 ± 5.8	0.2 ± 0.4 0.4 ± 0.4	40.7 ± 22.8 55.0 ± 10.3	8.4 ± 8.1 6.8 ± 8.5
	Diploma	57.3 ± 34.4 66.7 ± 65.5	276.4 ± 568.4 666.6 ± 467.6	103.0 ± 61.0 101.3 ± 119	69.5 ± 104.0 31.8 ± 125.1	5.3 ± 3.4 4.9 ± 1.06	0.4 ± 0.5 0.7 ± 0.6	29.7 ± 8.5 30.9 ± 13.3	0.5 ± 0.6 0.5 ± 0.6	44.8 ± 4.0 49.6 ± 24.8	4.7 ± 8.0 7.6 ± 13.8
	College	66.7 ± 23.2 73.6 ± 46.3	659.0 ± 557.0 367.8 ± 751.2	142.9 ± 184 100.5 ± 65.6	106.3 ± 96.3 65.6 ± 75.4	5.5 ± 5.9 5.1 ± 0.78	0.6 ± 0.5 0.6 ± 0.5	27.2 ± 8.5 35.3 ± 20.0	0.5 ± 0.4 0.4 ± 0.5	48.5 ± 17.6 56.0 ± 28.1	4.4 ± 10.8 5.0 ± 8.2
	p-value	0.94 0.26	0.76 0.18	0.19 0.79	0.86 0.41	0.65 0.15	0.21 0.93	0.08 0.26	0.61 0.51	0.11 0.50	0.50 0.69

Table 4. The levels of heavy metals (median ± IQR)* in relation to significant features between exposed and non- exposed to SHS groups. *Significant test in level 0.05, Mean ± standard deviations presented in First row is for non- SHS exposed group and Mean ± standard deviations presented in second row is for SHS exposed group, followed by a statistical comparison between the groups.

SHS, most trace elements had no significant correlation with the babies' weight, length and head circumference. Based on the results from multiple regression analyses as reflected in Table 6, exposure to SHS did not significantly impacted the serum levels of any trace elements.

Discussion

Numerous toxic and carcinogenic compounds in cigarette smoke enter the environment and are inhaled at home, workplace and other public areas. Exposure to cigarette smoke, both directly and indirectly, is detrimental to human health[18]. This study investigated the effects of SHS on pregnant women, an important and vulnerable group in the community. In general, our results demonstrated that passive smokers had higher serum concentrations of trace elements than those unexposed to SHS, although the differences between the two groups were insignificant, except for the effects on the newborns' growth indices. The findings of the current study suggest significant associations of trace elements with various birth metrics among the newborn babies of mothers exposed to SHS. Our results regarding the effects of trace elements exposure on the newborns' anthropometric features both agree with and differ from the existing literature in several aspects.

Earlier studies have shown that both active and passive smoking result in elevated serum blood levels of heavy metals in smokers compared to non-smokers[19–25]. Apostolou, et al.., have reported that higher number of smokers at home increases the Pb level in children's blood[26]. It has already been shown that the mean Pb level in indoor air has been higher in homes where smoking occurs than in those where no smoking occurs (21.8 ng/m3 vs. 7.8 ng/m3)[27]. A study conducted in Turkey has shown that children whose parents smoked had significantly higher levels of Cd, Pb, Cr, Sb, Fe, and Al in their hair samples compared to children of nonsmoking parents[28]. It is no surprise that exposure to tobacco smoke is linked to high levels of trace elements in the hair samples. Different brands of tobaccos and cigarettes contain varying amounts of toxic trace elements, such as As, Cd, Cr, Ni, and Pb, all of which known to be carcinogenic to humans[29–31].

The accumulative effects of trace elements in the body can be harmful to health over time. In the current study, most mothers reported their concurrent exposure to SHS. Thus, much attention should be paid to such metals, as Pb and Cd in the environmental air, especially because their half-life in humans are 10–12 years[32]. Exposure to other trace elements, such as As, Cd, and Ni even at low concentrations may lead to carcinogenic outcomes. Therefore, much attention should be paid to the trans-placental carcinogenesis induced by exposure to the trace toxic elements[33].

Our findings demonstrated that the serum levels of Cr and Ni in mothers exposed to SHS were significantly higher than those in the unexposed mothers even though the findings from the regression analyses on these metals were not necessarily alarming. The serum levels of Cr and Ni found in this study may suggest contaminations since they exceeded the expected serum values in the general population. The findings emphasize the need for further research on the subject. Consistent with our findings, Moradnia, et al., measured the urinary Cr and Ni levels in pregnant Iranian women, and suggested their association with lifestyle. Mean serum levels of Cr and Ni were significantly linked to passive exposure to cigarette smoke during pregnancy. This association may be due

Responses	Metals	Non-expose to SHS group			Expose to SHS group		
		Beta	SE	p-value	Beta	SE	p-value
Birth weight (gr)	Se	-0.002	0.007	0.765	-0.002	0.003	0.483
	Zn	0.0003	0.0004	0.540	-0.0003	0.0003	0.326
	Cu	0.001	0.002	0.622	-0.002	0.001	**0.016***
	Fe	-0.002	0.001	0.225	-0.001	0.001	0.319
	As	-0.103	0.068	0.139	0.016	0.043	0.710
	Cd	0.762	0.511	0.146	0.454	0.239	0.063
	Cr	-0.144	0.108	0.192	0.063	0.031	**0.044***
	Hg	0.186	0.389	0.636	0.254	0.245	0.303
	Ni	0.078	0.069	0.270	-0.034	0.023	0.147
	Pb	-0.029	0.038	0.447	0.004	0.016	0.790
Birth length (cm)	Se	0.001	0.023	0.958	-0.007	0.008	0.422
	Zn	-0.001	0.001	0.398	-0.002	0.001	**0.040***
	Cu	-0.003	0.006	0.661	-0.001	0.003	0.601
	Fe	-0.006	0.006	0.348	-0.002	0.003	0.436
	As	-0.039	0.231	0.867	0.209	0.133	0.121
	Cd	3.762	1.725	**0.036***	1.548	0.739	**0.041***
	Cr	-0.435	0.365	0.242	0.329	0.095	**0.001***
	Hg	2.453	1.316	0.071	-1.864	0.756	**0.017***
	Ni	0.269	0.234	0.258	-0.234	0.072	**0.002***
	Pb	0.002	0.130	0.984	0.112	0.051	**0.033***
Head circumference (cm)	Se	-0.056	0.020	0.009	-0.004	0.007	0.594
	Zn	0.001	0.001	0.644	0.001	0.001	0.241
	Cu	-0.001	0.006	0.868	-0.006	0.002	0.016
	Fe	-0.006	0.005	0.215	0.001	0.003	0.868
	As	-0.542	0.203	0.012	0.059	0.120	0.626
	Cd	1.956	1.517	0.206	0.484	0.665	0.469
	Cr	-0.310	0.321	0.342	0.253	0.086	**0.005***
	Hg	-0.068	1.157	0.953	0.590	0.681	0.389
	Ni	0.149	0.205	0.472	-0.140	0.065	**0.036***
	Pb	-0.092	0.114	0.429	0.080	0.046	0.093

Table 5. The result of relationship between infant characteristics and metals by multivariate regression. *Significant test in level 0.05.

Model	Metal (Response)	B	SE	*p*-value
1	Se (μg/l)	-0.100	0.162	0.539
2	Zn (μg/l)	-0.254	0.195	0.196
3	Cu (μg/l)	0.042	0.151	0.779
4	Fe (mcg/dL)	-0.093	0.306	0.762
5	As (μg/l)	0.021	0.083	0.799
6	Cd (μg/l)	0.168	0.226	0.458
7	Cr (μg/l)	0.121	0.078	0.123
8	Hg (μg/l)	0.249	0.222	0.265
9	Ni (μg/l)	0.105	0.077	0.174
10	Pb (μg/l)	0.347	0.346	0.318

Table 6. The effect of SHS exposure on trace element concentrations by multiple linear regressions. B: the coefficient of SHS exposure adjusted for other variables.

to various factors, including cooking utensils made of copper, aluminum, Teflon, and steel, as well as the use of cosmetics[34].

The general public is often exposed to Ni ions through breathing air, or consuming foods, and water. The adverse health effects of exposure to Ni include pulmonary fibrosis, contact dermatitis, and increased risk of cancers[35]. Nickel can easily cross the placental barrier and enter fetal blood and the amniotic fluid[35]. Developing organisms are particularly sensitive to a variety of toxins and carcinogens due to their rapid cell proliferation[33]. A former study conducted in 1992 has reported that Ni(II) is a potent trans-placental initiator of epithelial tumors in fetal rat kidneys and pituitary gland. Thus, it can be a major trans-placental carcinogen[36]. Other studies have suggested that Ni exposure may pose a significant risk of congenital malformations and prematurity[35,37]. A more recent study conducted in 2020 investigated maternal Ni exposure versus gestational length in a large birth cohort consisting of 7291 pregnant women in China[32]. The findings suggested that high level of Ni in the maternal urine was significantly associated with mildly reduced gestational age and increased risks of preterm delivery.

Chromium is a naturally occurring heavy metal found in the air, soil, water, and foods. Environmental Cr predominantly consists of two steady states of oxidation, i.e., trivalent (III) and hexavalent Cr (VI)[38]. Further, a higher risk of preterm birth is associated with increased maternal urinary level of chromium[38]. Chromium III is an essential trace element that originates from normal carbohydrate and lipid metabolism. This is the form that is available in most foods and nutritional supplements with very low risk of toxicity[39]. However, the nutritional necessity of chromium has recently been argued[40]. The hexavalent Cr is a heavy metal ion and is much more toxic than its trivalent counterpart. The International Agency for Research on Cancer has classified chromium (VI) as a carcinogen to humans through inhalation[41]. Both chromium versions (III &VI) can lead to adverse effects on fertility, reproduction, and the developmental processes of embryo in animals and humans[42–44].

Our study results showed that serum Cu levels are inversely related to birth weight in infants of exposed mothers. This is contrary to some studies suggesting that essential metals, such as Cu, are crucial in fetus development the deficiency of which is linked to lower birth weights[45]. Also, our study is in contrast with the findings of Oztan, et al.. who reported a positive correlation between Cu levels and birth weight[46]. However, other studies support our results, indicating that high Cu levels may adversely impact the normal fetal growth[47]. Özdemir, et al.. have suggested that increased Cu levels in both maternal and the cord blood may be associated with restricted fetal growth because of its adverse effects on the activity of the SOD1 enzyme[48]. On the other hand, Fahmey, et al.. did not find a significant relationship between serum Cu levels and body weight in the preterm neonates[49]. The discrepancy may suggest that the effect of Cu on fetal growth could vary by other contextual factors. There have been associations between high copper levels and the dysregulation of glucose metabolism during pregnancy. This can have consequences for fetal growth since glucose is a major energy source in fetus during development[50].

We found positive associations between Cr concentrations and birth weight, length, and head circumference in the mothers and SHS. A positive relationship between Cr and the above-mentioned birth parameters has been less often reported in the literature, so the potential toxic effect of high Cr levels remains a concern. A number of investigations have indicated that an increase in the levels of Cr in maternal urine samples at delivery and during pregnancy may result in a possible decrease in birth size and weight of the newborn[51,52]. However, these findings were not supported by other studies[53,54]. Our observations of reduced head circumference with a higher Ni level is supported by previous literature that linked heavy metal exposure to impaired fetal growth, particularly head circumference and birth weight[55]. In contrast, another study has reported that nickel concentration in the maternal urine did not affect the baby's birth weight[56].

Nickel is capable of crossing placental barrier thus inducing toxicity on embryonic tissues, resulting in reduced inner cell mass and trophoblast cell numbers[57]. The harmful effects of mercury on the birth length are consistent with studies that reported mercury may have adverse impacts on fetal growth through epigenetic modifications[58]. All of these findings suggest that the impact of trace elements on fetal development is complex and is likely to be influenced by a number of factors, such as maternal dietary intake and environmental variables. In addition, these findings underscore the importance of further research into the mechanisms whereby trace metals impact fetal development. From a practical perspective, they emphasize the monitoring and management of maternal exposure to trace toxic elements during pregnancy for optimal birth outcomes.

Limitations of the study
Despite the useful findings, this study had several limitations. Primarily, using a larger sample could add more generalizability to the findings and provide statistical power for unraveling linkage between SHS and trace elements in pregnant women. Although some confounding variables were controlled in this study, others, such as dietary patterns, socioeconomic status, lifestyle habits, and the subjects' comorbidities might have influenced the trace element levels and the developmental indices of the babies. Also, we did not incorporate specific assessments or controls for sources such as water, food, and other environmental exposures in the current study, which might have affected the interpretation of our finding.

Conclusions
Our findings provide evidence for possible developmental hazards toward the neonates in pregnant women due to passive exposure to SHS. Our findings demonstrated that SHS is associated with significant increases in the serum levels of Cr and Ni in pregnant women. However, the regression analysis results were not significant for the trace metal levels that could have been influenced by exposure to passive smoking.

The current study revealed complicated patterns of associations between the serum levels of trace elements and newborn growth indices. Among the mothers exposed to SHS, higher levels of Cu were negatively associated with their babies' birth weight. However, high Cr levels were positively linked to the birth weight, length, and head circumference. The elevated level of serum Ni was associated with reduced head circumference, and Hg affected newborn length negatively. Fewer associations were observed in the babies born to the non-exposed mothers. The results suggest the existence of complex interactions between SHS exposure, trace metal bioaccumulation, and fetal development.

This study has shown the dual effect of SHS exposure, where it may alter the serum levels of certain elements in pregnant women and contribute to growth abnormalities in newborns through yet unknown mechanism. Our findings emphasize the urgent need for preventive and educational programs to diminish SHS exposure of mothers during pregnancy, thus protecting the maternal and fetal health. Future studies should be conducted to examine the long-term effects of exposure to trace elements versus the maternal and the child health. Such research help elucidate the associations among factors that may affect pregnant women as they live in environments contaminated with cigarette smoke.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
KS, SN, BM, and AH generated the idea and design of the study. SN, BM, MA, and KT searched the literature in databases, and wrote parts of the manuscript. ZM participated in statistical analyses and edited the result part. KS, SN, BM, ZM, AH, MA, NEH, and KT reviewed the manuscript. KS, SN, BM, MA, NEH, and KT wrote the discussion section, and with KS and BM supervised all parts of the revision part of the manuscript. BM served as the corresponding author.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The study's protocols were reviewed and approved by Kermanshah University of Medical Sciences' Ethics Committee (Ethics code: IR.KUMS.REC.1402.510).

Also, each participating woman reviewed the study protocol and signed a consent form prior to being enrolled in the study.

Additional information

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