

## Lead Exposure and Serum VEGF and TGF- $\beta$ 1 Levels of Residents in Area with High Incidence of Cancer Along S River

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**【Abstract】 Objective:** This study was aimed to investigate the influence of lead pollution of S River on the serum VEGF (vascular endothelial growth factor) and TGF- $\beta$ 1 (transforming growth factor- $\beta$ 1) levels in residents of high cancer incidence areas. **Methods:** Contaminated and control area were chosen from villages less than 5km and more than 20km away from the S river respectively, which have similar population composition and economy condition. The concentrations of lead in drinking water, soil, grain and vegetables samples from two areas were measured respectively by flame atomic absorption spectrometry. A total of 796 residents aged from 30 to 60 years old were collected and divided into 4 groups including contaminated high-risk group, contaminated normal group, control high-risk group and control normal group via questionnaire. Serum lead levels were detected by cyclic voltammetry method. Serum VEGF and TGF- $\beta$ 1 levels were measured by ELISA. **Results:** The lead levels in drinking water, soil, vegetables and grain samples of contaminated area were higher significantly than those of control area respectively ( $P < 0.05$ ). The serum lead mean level of residents in contaminated area (115.82 $\mu$ g/L) was higher than those in control area (89.24 $\mu$ g/L) significantly ( $P < 0.05$ ). Serum VEGF and TGF- $\beta$ 1 levels of contaminated high-risk group were both higher than those of other three groups significantly ( $P < 0.05$ ). The serum VEGF and TGF- $\beta$ 1 levels in 4 group had obvious positive correlation ( $r = 0.79$ ,  $P < 0.001$ ). Serum VEGF and TGF- $\beta$ 1 levels varied between groups with different serum lead levels significantly. Serum VEGF and TGF- $\beta$ 1 levels were decreasing along with the increasing of serum lead levels when the serum lead concentration was lower than about 100 $\mu$ g/L, while when the serum lead concentration was higher than 110 $\mu$ g/L, serum VEGF and TGF- $\beta$ 1 levels were significantly increasing ( $P < 0.05$ ). **Conclusion:** The lead pollution of S river increases the lead exposure that may have relationship with the increasing of serum VEGF and TGF- $\beta$ 1 levels in residents of contaminated area with high mortality of tumor.

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**Key words:** Lead, Water Pollution, VEGF, TGF- $\beta$ 1, Cancer

### 1. Introduction

The S River is the largest branch of Huaihe River which is the third largest river in China. The pollution of Huaihe River has been reported because of the discharging of domestic sewage, industrial waste residue, and the over using of chemical fertilizer and pesticides in agriculture. The water quality of S River has been classified as Class V of surface water quality standards of China (Gao, 2010). Our previous study also proved that the mortality of liver cancer, rectal cancer, pancreatic cancer and lung cancer in S River contaminated area was significantly higher than that in control area (Li, 2011). In many environmental carcinogenic factors, lead and lead inorganic compounds have been classified by IARC (2012) as 2B and 2A. Exposure to lead is a possible or probably risk

factor of cancer. The average concentration of lead in the sediment of main stream of Huaihe River was 29.9 mg/kg (17.3~72.8 mg/kg) and 1.18 times of the average concentration of river sediment in China (Luo, 2010). However, the lead pollution of S river and surrounding residents' exposure to lead haven't been investigated.

Vascular endothelial growth factor (VEGF) is vital in the angiogenic process of human malignancies (Koukourakis, 2004). Transforming growth factor  $\beta$  (TGF- $\beta$ ) is secreted abundantly by tumor cells and the local microenvironment, which promotes invasion and metastases of various tumors. TGF- $\beta$  signaling blockade has proved to be efficient at preventing the development of a variety of tumor types (Delphine, 2011; Feltl, 2005). Both VEGF and TGF- $\beta$  are important factors in forming of cancer. However,

most previous reports of VEGF and TGF- $\beta$  focus on clinical cancer cases and data from population of health or premalignant condition is rare. The present study is planned to investigate the influence of lead exposure on the serum VEGF and TGF- $\beta$ 1 levels in residents of high cancer incidence areas, which may give clue to the further research of early diagnostic biomarkers for tumors.

## 2. Materials and methods

### Location

S country with S river flowing through was chosen as the survey scene. The contamination area and control area were selected randomly from the villages that less than 5 km and more than 20 km away from S river. Two villages were chosen by cluster sampling from the contaminated and control area respectively and the two villages have similar living habits, economic level, natural condition, demographic composition and so on. Details of the location can be found in Li Ping et al (Li, 2011).

### Subjects

All villagers aged from 30 to 60 years old in contaminated area and control area were recruited. According to high-risk factors such as family history of cancer, history of digestive system diseases, obvious gastrointestinal symptoms and smoking or drinking habit, subjects were divided into four groups, contaminated high-risk group, contaminated normal group, control high-risk group and control normal group. Each study subject was drawled 10ml fasting blood and the blood samples were centrifuged to separate serum and stored at -80°C for use.

### Questionnaire

The contents of the questionnaire includes general conditions, vocation and living behaviors, types of drinking water, fuel, dietary structure, subjective symptoms within two weeks and in recent six months illness, medical diagnosis and physical examination. Questionnaire was conducted as face to face interview survey by investigators who had been trained uniformly.

### Detection of lead concentration in environmental samples

Three river sections of S river, named as section 1, section 2, and section 3, were selected to collect the water samples as well as sludge samples in wet and dry season respectively. Samples of drinking water, soil, grain and vegetable were collected from five positions, east, south, west, north and central location of two villages chosen by cluster sampling from the contaminated and control area respectively. At each position, three samples were collected. The lead concentration was detected by direct absorption flame atomic absorption spectrometry. The each detection was parallel measured by two times and then calculated the average value.

### Measurement of serum lead, VEGF and TGF- $\beta$ 1 levels

After the Information Consent Form (ICF) was signed by all study subjects, 10ml fasting venous blood samples were collected and keeping still for 30 min, and then were centrifuged to separate the serum and stored at -80°C for use. The serum lead concentration was measured by cyclic voltammetry (Switzerland Wan Tong Co., Ltd China). The serum VEGF and TGF- $\beta$ 1 levels were measured by ELISA reagent kits purchased from Shanghai Yuan Valley Technology Development Co., Ltd. (China) and all measurement steps followed the kits' instruction.

### Statistical analyses

The questionnaire data was double entered into the database which was established by using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark) by different two people. The SPSS 12.0 software (SPSS Inc. 2003) was used to compare the lead mean concentrations in drinking water, vegetable and grain samples from contaminated and control areas by two independent sample *t* test. The SNK test of One-way Analysis Of Variance (ANOVA) was used to analyze the serum levels of lead, VEGF and TGF- $\beta$ 1 in 4 groups. Spearman's Rank Correlation Analysis was used to identify the correlation between VEGF and TGF- $\beta$ 1. The significant level is set at 0.05 ( $\alpha = 0.05$ ).

## 3. Results

### 3.1 Lead concentrations in environmental samples

The lead concentrations in river water samples and sludge samples taken from three river sections respectively were not significantly different shown in Table 1. A Total of 120 environmental samples were collected from the contaminated and control areas respectively. Of each kind of environmental samples, such as drinking water, soil, grain and vegetable samples, 15 samples were archived respectively in contaminated area and control area. Lead concentrations in drink water, soil, grain and vegetable samples of contaminated area were all higher significantly than those of control area ( $P < 0.05$ ) as shown in Table 2.

### 3.2 Serum lead, VEGF and TGF- $\beta$ 1 concentrations

796 villagers aged from 30 to 60 years old in contaminated area and control area were recruited and divided into 4 groups. The serum mean level of lead in residents of contaminated area was 115.82 $\mu$ g/L, which was higher significantly than that of control area, 89.24 $\mu$ g/L ( $P < 0.05$ ). In detail, the serum lead level of contaminated high-risk group, contaminated normal group, and control high-risk group were respectively higher than those of control normal group significantly ( $P < 0.05$ ) as shown in Table 3. Serum VEGF and TGF- $\beta$ 1 levels of contaminated high-risk group were higher significantly than those of control normal group,

contaminated normal group, and control high-risk group ( $P<0.05$ ). Serum VEGF and TGF- $\beta$ 1 levels of contaminated normal group and control high-risk

group were all higher significantly than those of control normal group ( $P<0.05$ ).

**Table 1. Lead concentration in water and sludge samples of S river in wet and dry season**

Groups	No.	Section 1 ( $\bar{x} \pm s$ )	Section 2 ( $\bar{x} \pm s$ )	Section 3 ( $\bar{x} \pm s$ )	F	P
water samples ( $\mu\text{g/L}$ )	27	75.30 $\pm$ 11.49	54.30 $\pm$ 5.49	74.00 $\pm$ 13.35	3.662	0.091
sludge samples (mg/kg)	27	132.27 $\pm$ 34.70	143.54 $\pm$ 52.43	171.55 $\pm$ 22.50	1.651	0.225

**Table 2. Comparison of lead concentrations in drink water, soil, grain and vegetable samples between contaminated and control area**

Groups	Contaminated Area		Control Area		t	P
	No.	Lead ( $\bar{x} \pm s$ )	No.	Lead ( $\bar{x} \pm s$ )		
Drinking Water ( $\mu\text{g/L}$ )	15	17.20 $\pm$ 8.31	15	7.20 $\pm$ 1.20	2.663	0.029
Soil (mg/kg)	15	184.80 $\pm$ 97.10	15	68.01 $\pm$ 52.28	-2.300	0.023
Grain (mg/kg)	15	0.33 $\pm$ 0.16	15	0.18 $\pm$ 0.11	-3.001	0.005
Vegetable (mg/kg)	15	0.08 $\pm$ 0.03	15	0.06 $\pm$ 0.02	-2.117	0.037

**Table 3. Comparison of serum lead, VEGF and TGF- $\beta$ 1 levels in 4 groups**

Groups	No.	Lead ( $\bar{x} \pm s$ ) ( $\mu\text{g/L}$ )	VEGF( $\bar{x} \pm s$ ) (ng/ml)	TGF- $\beta$ 1( $\bar{x} \pm s$ ) (ng/ml)
Contaminated high-risk group	201	117.40 $\pm$ 3.87 <sup>a#</sup>	1255.69 $\pm$ 59.85 <sup>a#*</sup>	907.46 $\pm$ 35.99 <sup>a#*</sup>
Contaminated normal group	193	111.10 $\pm$ 4.62 <sup>a</sup>	789.12 $\pm$ 44.57 <sup>a</sup>	386.33 $\pm$ 50.18 <sup>a</sup>
Control high-risk group	202	100.83 $\pm$ 3.27 <sup>a</sup>	792.87 $\pm$ 70.15 <sup>a</sup>	756.09 $\pm$ 60.91 <sup>a</sup>
Control normal group	200	86.97 $\pm$ 1.89	486.76 $\pm$ 39.00	269.76 $\pm$ 17.62

**Note:** 'a' compared with control normal group,  $P<0.05$ ; '#' compared with control high-risk group,  $P<0.05$ ; '\*' compared with contaminated normal group,  $P<0.05$ .

### 3.4 Relationship of serum VEGF and TGF- $\beta$ 1 concentrations in groups with different serum lead levels

Based on the serum lead concentration, all research objects were divided into 5 groups with <70, 70~, 90~, 110~ and  $\geq 130\mu\text{g/L}$  lead level in serum respectively. The serum VEGF and TGF- $\beta$ 1 levels varied significantly in the 5 groups as shown in Table 4 and Figure 1. After the correlation analysis, it was proved that serum VEGF and TGF- $\beta$ 1 concentrations had positive correlation as the correlation coefficients was 0.796 ( $r=0.796$ ,  $P<0.001$ ).

**Table 4. Serum VEGF and TGF- $\beta$ 1 concentration (ng/ml) in groups with different serum lead concentration**

Groups based on the serum lead concentration ( $\mu\text{g/L}$ )	No.	VEGF ( $\bar{x} \pm s$ )	TGF- $\beta$ 1 ( $\bar{x} \pm s$ )	r	P
< 70	157	940.11 $\pm$ 59.42	636.44 $\pm$ 43.62	0.796	<0.001
70~	118	797.63 $\pm$ 62.37	543.74 $\pm$ 47.50		
90~	168	474.45 $\pm$ 44.89 <sup>#</sup>	286.32 $\pm$ 20.91 <sup>#</sup>		
110~	255	779.23 $\pm$ 54.92 <sup>@</sup>	554.51 $\pm$ 39.20 <sup>@</sup>		
$\geq 130$	98	1292.36 $\pm$ 84.76 <sup>*\$</sup>	901.15 $\pm$ 75.13 <sup>*\$</sup>		
F		34.865	52.386		
P		< 0.001	< 0.001		

**Note:** '\*' compared with "<70" group,  $P<0.05$ ; '#' compared with "70~" group,  $P<0.05$ ; '@' compared with "90~" group,  $P<0.05$ ; '\$' compared with "110~" group,  $P<0.05$ .

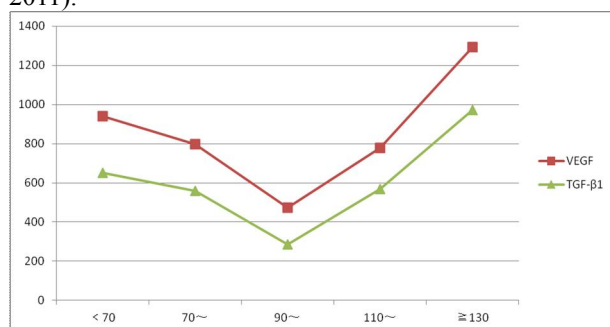
## 4. Discussion

### The lead pollution of S river and residents' exposure to lead

The heavy metal pollution of Huaihe River has been

reported and now the lead pollution of S River, the largest branch of Huaihe River, was observed as shown in Table 1-2. The lead concentrations of river water and sediments samples exceeded the class III and IV

standard (0.05 mg/L) of Surface Water Environmental Quality Standard of China (GB 3838-2002). No difference of the lead concentration among the samples from three river sections indicated the extent of pollution and the loss of the river's self-purification. The lead concentrations in drinking water, soil, grain and vegetable samples higher significantly in contaminated area than those in control area suggested the expansion of the lead pollution. It has been reported that the polluted river water filtered through the soil and soaked into the drinking water source (Charry, 2008; Tang, 2012). Then absorbed by plants, lead and inorganic lead compounds can be transferred from soil and water into grain and vegetable (Sharma, 2008; Muchuweti, 2006). There are several way by which residents living in contaminated area exposed to exceeded lead, such as intake of drinking water, food and other possible routes (Jan, 2010; Jan, 2011). In fact, the serum lead concentration of residents in contaminated area, 5 km away from S river, was significantly higher than that in control area as shown in Table 3. The exposure to exceeded lead may be related to the high incidence of cancer in the contaminated area as described in previous study (Li, 2011).



**Figure 1. Serum VEGF and TGF-β1 concentration ( ng/ml ) varied in groups with different serum lead concentration**

#### **Serum VEGF and TGF-β1 concentrations increased in contaminated area**

The serum VEGF and TGF-β1 levels were compared between contaminated area and control area to investigate the possible relation between lead pollution and cancer forming. High-risk group was designed in consideration of the confounding of other humor high-risk factors such as smoking, drinking and so on (Valavanidis, 2009). As observed in Table 3, the contamination factor only or high-risk factors only increased serum VEGF and TGF-β1 levels, which implied that pollutants including lead or high-risk factors had influence on forming of cancer, since serum VEGF and TGF-β1 levels of contaminated normal group and control high-risk group were all higher significantly than those of control normal group

( $P < 0.05$ ). Besides, comparison results suggested that pollution and high-risk factors may have synergistic action (Hines, 2010) in the process of cancer forming because serum VEGF and TGF-β1 levels of contaminated high-risk group were higher significantly than those of contaminated normal group, and control high-risk group ( $P < 0.05$ ). So the regular survey of residents with high-risk factors in contaminated area is necessary and valuable for their high cancer susceptibility.

#### **The extent of lead exposure influenced the serum VEGF and TGF-β1 levels**

Serum VEGF and TGF-β1 levels varied significantly in groups with different serum lead concentration. As shown in Table 4, when serum lead concentration increased from 70 μg/L, serum VEGF and TGF-β1 levels descended and went to the minima significantly with serum lead concentration rising to about 100 (90~) μg/L. However, with serum lead concentration over 110 μg/L, serum VEGF and TGF-β1 levels promoted rapidly. They went to the significantly higher level with serum lead level of 130 μg/L than those with serum lead level of 70 μg/L. The normal value of serum lead concentration is 100 μg/L in China, which is also the recommended level approved by WHO. The variation trend occurred in Figure 1 suggested that when serum lead level under or over the normal value, there may be two different toxic damage caused by increasing serum lead concentration. The secretion of VEGF and TGF-β1 may be inhibited by increasing serum lead concentration under the normal value. The hormesis (Martins, 2011; Calabrese, 2011) of initial increasing over the normal value of serum lead concentration may explain the recovery of serum VEGF and TGF-β1 levels before their significant increase. Further study is deserved to explore the complexity of different health damage and its mechanism with different extent of lead exposure.

A good correlation of serum VEGF and TGF-β1 levels was found in Figure 1, which was in accord with other study (Benkert, 2003). It could be explained by the interaction of VEGF and TGF-β1 in stimulating carcinogenic process. It gave an interesting clue to explore the tumor's early detection, such as joint detection of serum VEGF and TGF-β1 with more persuasiveness than single item.

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