Helicobacter Pylori Infection and Immune Factors On Residents in High-incidence Areas of Cancer Along S River

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Abstract: This study was conducted via questionnaire among the residents from 30 to 60 years old in two areas to explore the influence of water pollution on people's health condition. The Contaminated area situated less than 2 km away from S river whereas the control area was chosen at least 20 km away from the river. The subjects were divided into four groups according to family history of cancer, gastrointestinal diseases history, gastrointestinal symptoms and smoking or drinking habit, including high-risk group （group 1）and normal group（group 2）who lived in Contaminated area, high-risk group （group 3）and normal group（group 4）who lived in control area respectively. Immunoblotting method was applied to test HP antibodies in serum, levels of IL-2 and INF-γ in serum were measured by ELISA method.We found that type Ⅰ HP infections (CagA or VacA-positive) in group 1, group 2 and group 3 were all significantly higher than that in group 4 *(p<0.001),* while type Ⅱ HP infection (UreA or UreB positive) had no significant difference among groups *(p> 0.05)*，which points out that the residents living in the contaminated area along S River had significantly higher Helicobacter pylor. Values of IL-2 and INF-γ in group 1 was significantly higher than that in the other groups *(p <0.05)*, while levers of IL-2 in group 2 and group 3 were higher than group 4 *(p <0.05)*. Group 1 had a higher level of INF-γ than group 2 *(p <0.05),* these results indicated that the contaminated area residents along S River also had significantly higher immune factors . Both of which are risk factors of gastrointestinal cancer.

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1. Introduction

The Huai River Basin is the third largest river in China, which has a total length of 1000 km and covers an area of 270,000 km^2. About 150 million people are brought up by the Huai River. Since the 1980s, the water quality has been getting worse and worse because of the dramatic population growth, economic development, the rapid acceleration of urbanization and the large quantities discharging of domestic sewage, industrial effluent and agricultural pollutants. The most polluted section is S river, because various types of wastewaters from 31 cities were poured into the river without any sewage disposal. The monitoring (Wang etal, 2007; Gao etal, 2010; Tian etal, 2011) data showed that S River had accepted so many municipal and agro-industrial pollutants that it had already lost its self-purification capacity of environment, in which numbers of indicators had been classified as Class Ⅴ of surface water quality standards or even worse. Some researches (Xu, 2007) have already reported that water pollution of Huai River has caused great harm to the health of surrounding residents resulting in multiple high-incidence area of cancer around the basin. We had analyzed the cause of death in contaminated area and control area, and found that the proportion of people dying of cancer, accounted to 29.83%, was significantly higher in contaminated area， especially gastrointestinal cancer. The high-incidence areas of cancer assumed a focal distribution along S river and are consistent with the river pollution.

The effect of HP infection on health of people is a major issue of social concern. Many literature (Amieva etal, 2008; Takahashi etal, 2004) indicated that high incidence of gastrointestinal cancer related with HP infection. However, most of investigations were about clinical patients. It was reported rarely in normal population using strain typing method. There were also many scholars (Dimitrios etal, 2010; Mantovani etal, 2008; Hussain etal, 2007; Henson etal, 2011) who did believe cancer had a certain correlation with inflammation, yet mainly paid more attention to patients. In an early phase, the influence of Huai River pollution on the health of residents mainly focused on the incidence of cancer while less for the levels of

immune factors . Therefore, the objects of this study were designed to research HP infection and levels of immune factors (IL-2 and INF-γ) in serum of the residents between contaminated area and control area in order to explore the impact of water pollution on people's health condition and provide the scientific basis for selecting monitoring indicators.

2. Materials and Methods

Location

According to the data about disease spectrum and water monitoring from relevant health and environmental departments, and opinions of experts, S county which is the last place that S River fiows through in Henan Province was chosen as the survey scene for this cross sectional study. The polluted area and control area were selected randomly from the villages that less than 2 km and more than 20 km away from the river respectively. And the two villages both have 3,000,000 people and are similar in the economic level, natural condition, demographic composition and living habits and so on.

Subjects

Villagers aged from 30 to 60 years old who has been living in these two survey areas were recruited. Based on some elements such as family history of cancer, history of digestive system diseases, obvious gastrointestinal symptoms and smoking or drinking habit, they were divided into four groups: high-risk group （group 1）and normal group （group 2）who lived in Contaminated area, high-risk group （group 3）and normal group（group 4）who lived in control area respectively. Then 65 subjects were randomly selected in each group using random number table. Every subject was drawled 10ml fasting blood ,then the samples were centrifuged to separate serum and stored at -80℃ for use.

Analysis of Cause of Death

All data about a three-year period from 2007-01-01 to 2009-12-31 were obtained via direct reporting death registration network system of S County that included the entire population data, the list of all death, cause of death, death symptoms, treatment hospitals and diagnostic basis,etc. Household survey was used on the death list in order to further verify cause of death and diagnosis according to ICD-10 Classification of Diseases, while the demographic in 2009 of S County was used to calculate gender mortality, age-specific mortality and the cause of death.

Questionnaire

The questionnaire included villagers' general condition, vocational and life behavior, the types of drinking water and fuel, dietary structure, clinical symptoms, two weeks and six months of illness and so on. Investigators were trained in advance and the villagers were asked by face to face interview survey.

HP Infection Analyses

HP was tested by Immunoblotting, kits were purchased from Shenzhen Bo Lao te Biological Products Co., Ltd. The experiment was done according to steps on the introduction and then compared with the "standard zone" to judge results. There are totally three different results ① CagA or VacA antibodies simultaneously or either positive is type Ⅰ HP infection. ② only Ureas antibodies positive is type Ⅱ HP infection. ③CagA, VacA and Ureas all negative is Hp-negative.

Immune Factors in Serum Testing

IL-2 and INF-γ levels in serum were measured by ELISA. At the end of the measure in all samples, 10 samples which selected randomly were retested. kits were supported by Shanghai Yuan Valley Technology Development Co., Ltd.

Statistical Analysis

The data utilized double people enter into the database which was established using Epidata 3.0 software (Epidata 3.0 for windows, Epidata Association Odense, Denmark). HP infection, the top eight causes of death and the mortality of the top eight cancer were assessed using Fisher exact value method and $\chi 2$ test. The levels of immune factors (IL-2 and INF-γ) were summarized with descriptive statistics. One-way analysis of variance (ANOVA) test and SNK test were used to compare mean differences in four groups. SPSS17.0 statistical package was used for statistical analysis. (significance level $\alpha = 0.05$).

3. Results

3.1 The Top Eight Causes of Death in Contaminated Area and Control Area (Table 1)

Compared with control area, we can observe that the percentage of tumor and cerebrovascular disease was significantly higher ($P<0.05$), while others have no significant differences in two areas ($P>0.05$) (Table 1).

3.3 HP Infection in Four Groups

As was shown in table 3,Type Ⅰ HP infection (CagA or VacA-positive) in group 1 , group 2 and group 3 all were significantly higher than that in group 4 ($p <0.001$);while type Ⅱ HP infection (Ur eA or Ur eB positive) had no significant difference among groups ($p> 0.05$) .

TABLE 1. The top eight causes of death and their percentage（%）in two areas

	contaminated area		control area	
death cause	name	percentage（%）	name	percentage（%）
1	tumor	29.83*	tumor	13.22
2	cerebrovascular disease	23.05*	cerebrovascular disease	8.82
3	cardiovascular diseases	15.43	respiratory diseases	8.58
4	respiratory diseases	15.08	cerebrovascular diseases	7.43
5	accidental death	11.86	accidental death	5.57
6	infectious and parasitic diseases	1.36	infectious and parasitic diseases	0.81
7	endocrine, nutritional and metabolic diseases	1.19	endocrine, nutritional and metabolic diseases	0.58
8	digestive diseases	0.85	genitourinary diseases	0.46
	others	1.35*	others	54.48

Note.*p<0.05 compared with control area

3.2 The Mortality of the Top Eight Cancer in Contaminated Area and Control Area

Compared with control area, the mortality of liver cancer, Rectal cancer, Pancreatic cancer and Lung cancer in contaminated area was significantly higher (P<0.05).while others have no significant differences in two areas (P>0.05) (Table 2).

3.4 IL-2of Human Serum in Four Groups

As were shown in table 4, Values of IL-2 in group 1, group 2 and group 3 were all significantly higher than that in group 4 *(p <0.05)*. Values of IL-2 in group 1 and group 3 were significantly higher as compared with group 2 *(p <0.05)*. Group 1 had a higher level than group 3 *(p <0.05)*.

TABLE 2. Comparison of the mortality of the top eight cancer in two areas (1/100, 000)

name	Contaminated area	Control area	$\chi 2$	P
Esophageal cancer	25.9	16.2	1.999	0.157
liver cancer	45.4	18.2	10.582	0.001*
Rectal cancar	13.0	4.1	4.321	0.038*
Pancreatic cancer	7.8	0.0	5.569	0.018*
Gastric cancer	22.0	18.2	0.315	0.574
leukemia	6.5	3.0	0.499	0.480
Breast cancer	11.7	4.1	3.398	0.065
Lung cancer	66.1	26.3	15.645	0.000*

Note.*$p<0.05$ indicating statistical significance of difference

TABLE 3. Comparison of HP infection in serum of four groups in two areas

groups	type Ⅰ HP infection	type Ⅱ HP infection	negative infection	No
1	61*	4	0	65
2	56*	2	7	65
3	60*	2	3	65
4	36	6	23	65
total	213	14	33	260

Note.*p<0.05 compared with group 4.

TABLE 4. Values of IL-2 in serum of four groups in two areas

groups	NO	IL-2($\bar{x} \pm s$)（pg/ml）
1	65	1006.455±146.603*a
2	65	608.060±144. 056#
3	65	754.570± 83.021#a
4	65	312.881±16. 881#a

Note. a p<0.05 compared with group 2, *p<0.001, # p<0.05compared with group 4.

3.5 INF-γ of Human Serum in Four Groups

As shown in table 5, Values of INF-γin group 1 and group 3 were all significantly higher than that in group 4 *(p <0.05)*; and group 1 had a higher level than group 2 *(p <0.05*).

TABLE 5. Values of INF-γ in serum of four groups in two areas

groups	NO	INF-γ($\bar{x} \pm s$)（ng/ml）
1	65	154.991±27.210*#
2	65	81.192±21.999
3	65	125.076±19.989*
4	65	60.231± 8.896

Note. # p<0.05 compared with group 2, *p<0.05compared with group 4.

4. Discussions

The cause of death has been influenced by S River Pollution

In recent years, according to the monitoring data, it showed that about 50% of the Huai River Basin were exceeding class Ⅴ standards of surface water quality in 2004 and it can directly affects the local people who live around the Basin (Wang etal, 2007). More and more attention had been paid to the incidence of cancer.

The results of this study showed that: 1) the percentage of tumor and cerebrovascular disease was significantly higher (P<0.05). 2) the mortality of liver cancer, Rectal cancer, Pancreatic cancer and Lung cancer in contaminated area was significantly higher (P<0.05).while others have no significant differences in two areas.

The research indicated that the proportion of people in contaminated area dying of cancer accounted to 29.83% and was significantly higher , especially gastrointestinal cancer. It suggested that the cause of death has been influenced by the polluted river spread horizontally.

HP Infection in Contaminated Area and Control Area

HP was divided into two types in clinic : type Ⅰ HP, producing cytotoxic strain, can be easy to cause stomach diseases. Conversely, people have no clinical symptoms when suffered type Ⅱ HP which is less toxic. HP infection is common in crowd. And HP is not only the main cause for chronic gastritis and ulcer, but also closely related with gastric cancer and gastric mucosa-associated lymphomas (Amieva etal, 2008; Takahashi etal. 2004). Wang kJ, etc, (Wang and Wang 2003) conducted a Meta-analysis on the epidemiology of HP infection in China and indicated that HP widely distributed in China and its average infection rate was 58.07%. According to the research of Huang RG, etc, (Huang etal, 2011) the results had showed that HP infection and gastric disease history had a closely relationship.

The results of this study showed that 1) HP infection was lowest in group 4 in which type Ⅰ HP infection was 55.2% and consistent with the level of our national. 2) type Ⅰ HP infection in group 1, group 2 and group 3 all were significantly higher than that in group 4 *(p <0.001)* and the average level, Which is consistent with the disease spectrum data from health departments of S county showing that the incidence of cancer especially gastrointestinal cancer in contaminated area is much higher than that of the national average.

The Levels of Immune Factors in Contaminated Area and Control Area

IL-2 and INF-γ, important immune factors, can lead to immune tolerance (Michele etal, 2008; Robert etal, 2011) by affecting regulatory T cells via siginal transformation. Some researches indicated that tumors proned to happen if the immune function was inhibited as levels of IL-2 and INF-γ increased. Previous researches (Du etal, 2004) also had showed IL-2 in patients with gastrointestinal cancer was significantly higher than that in non-cancer group.

The result of this study showed that ① the levels of IL-2 and INF-γ in high-risk groups were significantly higher than that in normal groups *(p <0.05)*, ② group 1 has a higher IL-2 level than group 3 *(p <0.05)*. ③the levels of IL-2 and INF-γ in group 1 were significantly higher compared with that in group 4. ④ there is no statistically difference of levels of IL-2 and INF-γ between group 2 and group 3 *(P> 0.05)*.

According to the above results, it demonstrated that both polluted factors and other risk factors can together lead to levels of IL-2 and INF-γ significantly changing, so did the polluted factors only, which has some necessary link with the pollutants which affect human immune function in S River.

Recently some data (Wang etal, 2006) have shown high prevalence of type Ⅰ HP can reduce the function of immune system, meanwhile the reduce of immune factors conversly add the incidence of HP infection. They are high risk factors of all gastrointestinal tumors and may have some combined effect.

This study has indicated that the residents living in the contaminated area along S River had significantly higher Helicobacter pylori infection and immune factors, both of which are risk factors of gastrointestinal cancer. Therefore, residents living along S River can adopt some measures including early prevention and treatment of HP infection, and improving their immune function to reduce the incidence of cancer to improve the quality of life. At the same time, HP infection and immune factors can be used as monitoring indicators in the evaluation on effect of pollution control to obtain residents' attention.

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