Determination of grain contamined by *Alternaria alternata* and exposure of its toxins for residents in the high incident area of esophageal cancer

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Abstract

Objective. To investigate the exposure levels of *Alternaria alternata* toxins and evaluate the prevention effects for esophageal cancer in the high incident area of Linxian, China. *Methods*. *Alternaria alternata* toxins in grain and urine were measured using thin-layer chromatography. The current exposure levels were compared with the previous exposure data. *Results*. The contamination rates of *Alternaria alternata* in wheat were 12.05% and 38.50% for the samples in 2004 and 1981, respectively. The positive rates of alternariol (AOH) in wheat in 2004 (10.83%) was significantly lower than that in 1995 (21.74%). The positive rates of AOH were 10.83% in wheat grain and 3.33% in wheat flour. The AOH and alternariol monomethyl ether (AME) positive rates in urine in 2004 (4.04% of AOH, 1.01% and of AME) were all significantly lower than those in 1992 (17.50% of AOH and 7.50% of AME). *Conclusion*. The exposure levels for AOH and AME in grain and urine samples from the residents are significantly lower in 2004 than that in 1981 or in 1995 in Linxian of China. This indicates that the prevention measures for esophageal cancer are effective, and might contribute to the decrease of esophageal cancer in the area. [Life Science Journal. 2007; 4(4): 25 – 28] (ISSN: 1097 – 8135).

Keywords: esophageal cancer; urine; grain; *Alternaria alternata*; alternariol; alternariol monomethyl ether

1 Introduction

It has been shown in our previous investigations that contamination intensity of *Alternaria alternata* was much higher in the grains, especially in wheat, in the area with a higher incidence of esophageal cancer in 1980s[1]. Thereafter, studies have demonstrated *Alternaria atlternata* extracts from grains in Linxian could induce DNA mutation, oncogene activation, and special damage for the epithelial cell of esophagus in diverse cell types including epithelial cells of hominine embryo esophagus, and result in tumor in rats[2–7]. Moreover, *Alternaria alternata* was also shown to promote synthesis of MBzA (N-nitrosomethylbenzylamine) that specifically induce esophageal cancer[8]. Chemical analysis suggested that the effective components of *Alternaria alternata* causing hereditary damage were alternariol (AOH) and alternaiol monomethyl ether (AME)[9]. It has been found that the grains and urine from Linxian residents contained high levels of AOH and AME[10,11]. Epidemiological investigations have shown that there is a direct relation between the rate of esophageal cancer incidence and the exposure levels to *Alternaria alternata* in Linxian. Thereby, it has been proposed that reduction of grain fungus contamination might decrease the probability of esophageal cancer. The current study is to investigate the *Alternaria alternata* contamination in grains and the exposure levels of its toxins in urine in Linxian, so that to evaluate the efficacy of prevention measures for esophageal cancer.

2 Materials and Methods

2.1 Collection of samples

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The grains for isolation and culture of *Alternaria alternata* were obtained from the east, west, south, north, and middle areas of Linxian. Fifty samples of wheat and 50 samples of corn were collected at each site. One hundred and twenty samples of the flour from wheat and corn, and 120 samples of grain of wheat and corn were collected for measuring *Alternaria alternata*. In addition, 50 urine samples were also collected for *Alternaria alternata* toxin determination. The sampling sites were the same to those collected in 1981, 1992, and 1995.

2.2 Isolation and culture of *Alternaria alternata*

The isolation and culture methods have been described previously[1]. After sterilization, the grains were inoculated on the Czapek'solution agar and potato dextrose agar (PDA, made in our labortory) substrate, and then cultured at 26 ºC for 4 – 6 days. Preliminary screen of the cultures was conducted according to colony growth, and the suspicious positive colonies were excluded. The species of *Alternaria alternata* were classified according to Neergaard[10].

2.3 Examination of AOH and AME in grains

AOH and AME (Sigma, USA) were determined following Lou's method[11]. Specifically, 500 g of the grains were comminuted and mixed with petroleum ether. The flour was directly mixed with petroleum ether. Then the mixture was filtrated. The rest was mixed with ether again and extracted for 3 times. The extracts were collected, dried by volatilization. The fixed quantity of the extract was dissolved with 10 ml methyl alcohol. The solution was dripped on the silica gel plate and outspread with the system (toluene : acetic ether : formic acid = 5 : 4 : 1, v/v). The silica gel plate was dried with blower, and the color of the fleck and the value of R_f observed under ultraviolet radiation at 365 nm. The results were compared with those of the standard samples of AOH and AME (Sigma, USA)

2.4 Examination of AOH and AME in urine

Based on Lou's method[12], 0.5 – 1.0 L of urine was mixed with 150 – 300 ml ether, surged and extracted for 3 times. The extracts were collected, dried in the air. The fixed quantity of the extract was dissolved with 10 ml methyl alcohol. The solution was dripped the plate outspread. The observation and estimation of the results were carried out as described previously.

2.5 Statistical analysis

χ^2 test was used for comparing the differences of the positive rates between two groups. All the analyses were performed by SAS version 9.13 (SAS Institute Inc., Cary, NC). Statistical significance was defined as $P < 0.05$.

3 Results

3.1 Differences of *Alternaria alternata* in grain

As shown in Table 1, the positive rate of *Alternaria alternata* in wheat was significantly lower in 2004 than that in 1981 ($P < 0.01$). The rate of corn was 1.64% in 2004 and 2.43% in 1981, but the difference wasn't of significance between them ($P > 0.05$).

3.2 Differences of *Alternaria alternata* toxins in grains

The positive rates of AOH in wheat in 2004 (10.83%) was significantly lower than that in 1995 (21.74%) ($P < 0.05$). The rates were not different between AOH in corn and of AME in wheat samples ($P > 0.05$) (Table 2).

3.3 Differences of *Alternaria alternata* toxins in the flour and grains

The positive rates of AOH were 10.83% in wheat grain, higher than 3.33% in wheat flour ($P < 0.05$). But the rate of corn grain was not statistically different from corn flour. Similarly, difference of AME positive rate in wheat grain and flour was of no signifcance and corn grain and flour wasn't either ($P > 0.05$)(Table 3).

3.4 Differences of *Alternaria alternata* toxins in urines from Linxian

Table 4 showed that there were four AOH positive samples and one AME positive sample in 99 samples of urine from the residents of Linxian. The AOH and AME positive rates in urine samples in 2004 were all significantly lower than those in 1992 (AOH: 4.04% *vs.* 17.50%, $P < 0.01$; AME: 1.01% *vs.* 17.50%, $P < 0.01$).

4 Discussion

In order to investigate the exposure levels of *Alternaria alternata* in the grains and its toxins in human bodies, to evaluate the efficacy of preventing esophageal cancer in Linxian, this study was conducted in the following 4 aspects: 1) compared the contamination rates of *Alternaria alternata* isolated from grains in Linxian with those data in 1981. 2) Compared the *Alternaria alternata* toxins from grains in Linxian with those determined in 1995. 3) Compared *Alternaria alternata* toxin of flour in Linxian with those analyzed in 1995. 4) Compared *Alternaria alternata* toxin in urine of residents in Linxian with those determined in 1992.

Table 1. Differences *Alternaria alternata* in grain from Linxian between 2004 and 1981

Grains	The results in 2004			The results in 1981		
	Inoculation (*n*)	contamination (*n*)	contamination rate (%)	Inoculation (*n*)	contamination (*n*)	contamination rate (%)
wheat	2016	243	12.05*	1800	693	38.50
corn	2016	33	1.64**	2100	51	2.43

vs. the result in 1981, *χ^2 = 359. 28, *P* = 0. 001; **χ^2 = 3.225, *P* = 0. 073

Table 2. Differences in isolation and culture of grain *Alternaria alternata* toxins

Toxins	Wheat						Corn					
	Results in 2004			Results in 1995[11]			Results in 2004			Results in 1995[11]		
	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)
AOH	120	13	10.83	115	25	21.74*	120	4	3.33	45	4	8.89#
AME	120	11	9.17	115	19	16.52**	120	3	2.50	45	3	6.67##

vs. the result in 2004: *: χ^2 = 5. 152, *P* = 0. 023; **: χ^2 = 2.853, *P* = 0. 091; #: χ^2 = 2.189, *P* = 0.139; ##: χ^2 =2.189, *P* = 0.139.

Table 3. Differences in *Alternaria alternata* toxins in the flour and grains

Toxins	Wheat						Corn					
	Grain			Flour			Grain			Flour		
	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)
AOH	120	13	10.83	120	4	3.33*	120	4	3.33	120	2	1.67#
AME	120	11	9.17	120	9	7.50**	120	3	2.50	120	3	2.50##

vs. the grain: *: χ^2 = 5. 128, *P* = 0. 024; **: χ^2 = 0.218, *P* = 0. 640; #: χ^2 = 0.648, *P* = 0.408; ##: χ^2 = 0.000, *P* = 1.000

Table 4. Differences in *Alternaria alternata* toxins in urines from Linxian

Toxins	Results in 2004			Results in 1995[11]		
	samples (*n*)	positive (*n*)	positive rate (%)	samples (*n*)	positive (*n*)	positive rate (%)
AOH	99	4	4.04	40	7	17.50*
AME	99	1	1.01	40	7	17.50**

vs. the result in 2004: *: χ^2 = 7.082, *P* = 0. 008; **: χ^2 = 14.282, *P* = 0. 001.

These results indicated that: 1) The rates of contamination of *Alternaria alternata* in wheat and corn were 12.05% and 1.64 %, respectively in 2004. They were both lower than those in 1981 (38.50% of wheat and 2.43% of corn, respectively), especially in wheat. It suggests that the contamination of *Alternaria alternata* in grains in Linxian has been improved remarkably. 2) The toxins of *Alternaria alternata* from 120 samples of wheat and 120 samples of corn were examined in 2004. The results showed that the positive rates of AOH and AME in wheat and corn were all lower than those in 1995 at different extent, and the difference in AOH was highly significant in wheat, which indicates that the levels of AOH and AME which have marked genetic toxicity reduced significantly in main grains in Linxian. 3) The difference between AOH and AME levels in grains of wheat and corn and that of their flour showed that the positive rates of AOH and AME in the majority of the flour samples were lower than those in grain samples, and there was significant difference between the positive rates of AOH in the flour of wheat and in the grain of wheat. The exposure to *Alternaria alternata* for local residents reduced dramatically because of the distinct decrease of the toxins of *Alternaria alternata* in flour which is the main food directly taken by local residents. 4) Four out of 99 samples of urine of residents in Linxian contained AOH, and 1 out of 99 samples had AME in 2004. The results were all significantly lower than those in 1992. The toxin levels of *Alternaria alter-*

nata in urine not only directly indicate that the level of excretion from human body, but also indirectly reflect the existing levels in human body. The toxin levels in urine were the direct index on exposure of human body to the toxins of *Alternaria alternata* and the extent. The decrease of the toxins in urine observed in this study was consistent with the reduced *Alternaria alternata* contamination and toxins.

The results as depicted previously show that the contamination extent of *Alternaria alternata* and its toxins in main grains and existing level in the body of residents from Linxian have all reduced remarkably compared to those of last century from 1980s to early 1990s[1,11,12]. It was assumed that the causes of the reduction of *Alternaria alternata* and its toxins might be follows: 1) The period of harvest was shortened due to application of mechanization of crop harvest, and the storage condition of grains has been improved distinctly, so the contamination on grains are reduced. 2) The amelioration of grain breed has enhanced the crop's ability against the pathogen. 3) The *Alternaria alternata* mainly affects grains at the waxiness layer in bran and the embryo, and produces toxins locally. The local residents had the tradition that they seldom got rid of bran when processing grain. Thus there was no remarkable difference on toxin level between grains and flour. In the lastest 10 years, this tradition for the local residents has improved greatly, and grains are refined before processing. By getting rid of large amount of bran in grain processing, the toxins of *Alternaria alternata* in flour decrease remarkably.

5 Conclusion

This study suggests that a notable parallel relationship existed between the reduction of the exposure levels to *Alternaria alternata* toxins and the decreasing trend of the incidence of esophageal cancer in Linxian. This indicates that the prevention measures for esophageal cancer are effective, and might contribute to the decrease of esophageal cancer in Linxian of China.

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