

Intake of Volatile N-nitrosamines and Their Ability to Exogenously Synthesize in the Diet of Inhabitants from High-risk Area of Esophageal Cancer in Southern China¹

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Objective Nan'ao County in Guangdong Province is a high-risk area of esophageal cancer in Southern China. Of the suspected etiologic factors in the environment, N-nitrosamines and their precursors have received the greatest attention. **Methods** Sixty samples of the diet ingested by the inhabitants were collected and detected for volatile N-nitrosamines and their precursors. Five N-nitrosamines detected by Gas Chromatography-Thermal Energy Analyzer were N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitrosopyrrolidine, N-nitrosopiperidine and N-nitrosomethyl-benzylamine. **Results** The average content of 5 volatile N-nitrosamines in the diet was 312.0 µg/kg (median). The daily intake of the nitrosamines was 286.5 µg/head/day. Only the ability to exogenously synthesize N-nitrosopiperidine was powerful among 5 volatile N-nitrosamines. By a computerized stepwise regression analysis and curve fitting, we studied the correlation among the nitrosamines, the precursors and the major food items in the samples. **Conclusion** It demonstrated that a relatively high content of volatile N-nitrosamines was present in the diet collected in the area.

Key words: Volatile nitrosamines; Intake; Exogenous synthesis; Esophageal cancer

INTRODUCTION

Nan'ao County in Guangdong Province is known as a high-risk area of esophageal cancer in Southern China^[1]. Its standardized mortality rate of esophageal cancer is 110/10⁶/year, seven times as high as the average mortality rate of esophageal cancer in China. Nan'ao has its special geographic environment as it is an island, and its unique diet habit is significantly different from that in the high-risk areas of esophageal cancer in Northern China. N-nitroso compounds (NOC) are proved to be a sort of strong carcinogen in animals^[2,3]. The previous studies found that NOC may play an important part in the etiology of esophageal cancer in Northern China^[4,5]. But there still are lacks of confirmed evidence.

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We believe that more light should be shed on the NOC effect on the etiology of esophageal cancer in Southern China. Therefore, in this survey, we detected the level of volatile N-nitrosamines (VNOC) and its precursors, abilities to exogenously synthesize volatile NOC in the diet of inhabitants from Nan'ao County. In addition, the association among N-nitrosamines, its precursors and the major food items was also studied.

MATERIAL AND METHODS

Chemicals

Chinese Academy of Medical Sciences provided 6 volatile nitrosamines used as standards. The standard volatile nitrosamines were N-nitrosodipropylamine (NDPA), N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosopyrrolidine (NPYR), N-nitrosopiperidine (NPIP) and N-nitrosomethyl-benzylamine (NMBZA). The other reagents were commercially available and used without further purification.

Study Subjects and Sample Collection

By means of a sample procedure of 3-stage random cluster, 60 healthy male subjects (35-64 years old) and their diet were selected from Nan'ao County. Two townships were selected from the county. Two villages were selected from each township. Fifteen subjects were selected from each village. Then the inquiry about the lifestyle characteristics was made among the subjects.

Three meals of the subjects in a single day were collected. The diet was prepared in duplicate with one portion consumed and the other kept for analysis. The weights and items of food in the two portions were the same, and were measured and recorded. Ten mL of 20% ammonium sulfamate (in 1.8 mol/L sulfuric acid) was added to 1/10 part of the evenly mixed food and the sample was frozen at -30°C until analysis.

Analytical Methods

Gas Chromatography (GC)-Thermal Energy Analyzer (TEA) was used for the analysis of volatile NOC in the mixed foods of three meals^[6,7]. The mixed food (20 g), with 150 ng NDPA and 10mL mineral oil was distilled under vacuum at 120°C for 40 min. The distillate was extracted by dichloromethane and then concentrated to 0.5 mL on a Kuderna-Danish evaporator at 50°C for the analysis. The GC-TEA method was applied to detect NDMA, NDEA, NDPA, NPYR, NPIP and NMBZA. For GC (HP 5890, Hewlett Packard Co. U.S.A.), a stainless steel column (2m \times 2 mm i. d.) packed with 5% FFAP on Chromosorb W (80-100 mesh) was used, the temperature of the injection port of GC was 220°C , the original oven temperature was 100°C for 2 min, the increased rate of temperature was $10^{\circ}\text{C}/\text{min}$, the final temperature was 200°C for 5 min, the flow rate of the N_2 carrier gas was 30 mL/min. For the TEA (TEA, model 543, Thermedic Waltham, MA, U.S.A.), the temperature of pyrolyzer was 50°C , interface 200°C , vacuum to 1.0 mmHg. The average recovery was 81%. Another 25 g a ken from the same sample with 100 mg NaNO_2 was cultivated at 37°C for 4 h, then treated and detected by the same method as the above sample. In addition, 20 g a ken from the same sample was soaked in distilled water at room temperature overnight. Its filtrate was applied to Cadmium column. The total amount of nitrite and nitrate (NO_x^-) in the food was determined according to the method of Sen and Donaldson^[8].

Because the distribution of measured levels of VNOC was skewed, median and 95% confidence interval (95% CI) were used for the descriptive indexes. To compare the differences at cultivation beginning with at the end, X^2 -test and t -test for pairing data were used respectively for significance test of rates and averages. By a computerized stepwise regression analysis, curve fitting for 25 curvilinear kinds, the correlations among the VNOC, the precursors of VNOC and the major food items were analyzed^[9]. The calculations were carried out using the statistical package of SPSS 10.0 for windows. The daily intake of VNOC equaled the product of VNOC content and total intake of food in the three meals a day.

RESULTS

Contents and Intakes of VNOC and NO \bar{x} in Nan'ao Diet

Five volatile N-nitrosamines—NDMA, NDEA, NPYR, NPIP and NMB₂A, were found in the diet samples of inhabitants from Nan'ao County (Table 1). Among the VNOC, both the content and daily intake of NDMA were the highest. The medians of content and daily intake of NDMA were 221.0 $\mu\text{g}/\text{kg}$ and 224.9 $\mu\text{g}/\text{head}/\text{day}$, respectively. NDMA was detected in 93% samples (detectable rate 93%). The contents and intakes of NPYR and NPIP were all lower than those of other VNOC. The results of NO \bar{x} determined in the diet samples were as follow: the content median 8.5 mg/kg with 95% CI 2.4–35.8 mg/kg, the daily intake median 7.3 mg/head/day with 95% CI 1.7–41.2 mg/head/day.

TABLE 1

Contents and Daily Intakes of VNOC in the Diet of Inhabitants From Nan'ao County

VNOC	Detectable Rate (%)	Content ($\mu\text{g}/\text{kg}$)		Intake ($\mu\text{g}/\text{head}/\text{day}$)	
		Median	95% CI	Median	95% CI
NDMA	93	221.0	395.7 \pm 1102.5	224.9	385.5 \pm 1135.3
NDEA	84	78.0	102.9 \pm 286.7	66.7	96.6 \pm 280.4
NPYR	31	0.1 ^{DL}	0.1 \pm 3.7	0.1 ^{DL}	0.1 \pm 3.2
NPIP	33	0.1	1.2 \pm 2.8	0.1	0.8 \pm 2.6
NMB ₂ A	93	21.0	28.6 \pm 73.2	19.3	25.9 \pm 70.5
Total	98	312.0	566.5 \pm 1420.3	286.5	551.8 \pm 1448.6

Note. No. of subjects was 60; VNOC, volatile N-nitrosamines were determined by Gas Chromatography-Thermal Energy Analyzer; Nan'ao County, with the standardized mortality 110/10⁶/year, is known as a high-risk area of esophageal cancer in Southern China; NDMA, N-nitrosodimethylamine; NDEA, N-nitrosodiethylamine; NPYR, N-nitrosopyrrolidine; NPIP, N-nitrosopiperidine; NMB₂A, N-nitrosomethyl-benzylamine; 95 % CI, 95% confidence interval; DL, detection limit.

Associations Among Different VNOC in Nan'ao Diet

In the diet samples, the contents of NMB₂A and NDEA were found to correlate positively with that of NDMA (Table 2). There was also a positive correlation between contents of NPIP and NPYR. The results came from curve fitting and stepwise regression analysis. The variables of regression analysis were the contents of each VNOC in the samples.

TABLE 2

Associations Among the Contents of Different Volatile N-nitrosamines in the Diet of Nan'ao Inhabitants

Best Regression Equation	Decision Coefficient (R^2)
$NDMA=0.05+1.82 \times NDEA+6.66 \times NMB_2A$	0.50
$NDEA=0.49 \times NDMA-0.06 \times NDMA^2$	0.56
$NPYR = 135.30 \times NPIP^2 - 0.66 \times NPIP$	0.82
$NMB_2A=0.05 \times NDMA^{0.57}$	0.64

Note. The diet was the mixed foods of subjects from the three meals a day; The results came from the curve fitting and stepwise regression analysis; The variables were the contents of each volatile N-nitrosamine in the diet; Unit of the content was $\mu\text{g}/\text{kg}$.

Associations Among $NO\bar{x}$ and Different VNOC in Nan'ao Diet

After the regression analyses of single factor for 25 curvilinear kinds were made, any linear or curvilinear regression relationship was not found between $NO\bar{x}$ contents and contents of each VNOC in diet samples. By the computerized stepwise regression analysis, the interactions of multiple factors among variables of $NO\bar{x}$ and 5 VNOC were studied. When NDMA, $NO\bar{x}$, NDEA and NMB_2A were present simultaneously in diet samples, NDMA contents (dependent variables) were affected by other three independent variables. The regression equation of these variables was $NDMA=-0.47+2.13 \times NO\bar{x} + 1.91 \times NDEA + 7.05 \times NMB_2A$ and significant ($P<0.01$). Its multiple correlation coefficient (R) and residual standard deviation were 0.7604 and 0.9344, respectively. There was a synergistic action among variables of $NO\bar{x}$, NDEA and NMB_2A . These three variables in the equation were all significant ($P<0.01$). When the three variables existed simultaneously, they had similar effect on NDMA content, but the independent action of NDEA was the biggest among them.

Ability to Exogenously Synthesize of VNOC in Nan'ao Diet

Table 3 shows the determined results of VNOC in the diet samples after cultivation with 100 mg $NaNO_2$ at 37°C for 4 h. The detectable rates and contents of VNOC in the samples at the beginning of cultivation were respectively compared with that at its end. The detectable rate of NPIP was higher at the cultivation end than at the beginning ($\chi^2=6.0357$, $P<0.025$). The content of NPIP increased significantly at the cultivation end ($t=2.5923$, $P<0.05$ vs. the beginning of cultivation). Although the detectable rate of NPYR increased at the cultivation end ($\chi^2=7.2593$, $P<0.01$ vs. the beginning of cultivation), its content had not changed significantly. From the beginning to end, the detectable rates and contents of NDMA, NDEA and NMB_2A were all same.

Associations Among Food Items and VNOC Contents in Nan'ao Diet

The food items recorded were classified into the 4 categories of greengrocery, animal food, salted food and fried food. Then the stepwise regression analysis of the food items (independent variables —X) and the contents of each VNOC (dependent variables —Y) were made in the samples. The aim was to study the associations among different food items and VNOC contents in the diet of inhabitants from the high-risk area of esophageal cancer in Southern China. The computer sifted out greengrocery and fried food respectively from 4

independent variables. There was a significantly negative correlation of greengrocery with NPYR contents (correlation coefficient $r = -0.2907$, residual standard deviation 0.072, significance for r : $0.01 < P < 0.05$). There was also a significantly positive correlation of fried food with NMB₂A content ($r = 0.3761$, residual standard deviation 0.0824, $P < 0.01$).

TABLE 3

Determined Results of Volatile N-nitrosamines in the Diet Samples at the Cultivation End *in vitro*

Volatile Nitrosamines	Detectable Rate (%)	Content ($\mu\text{g}/\text{kg}$)	
		Median	95% Confidence Interval
NDMA	89	232.0	361.6 ± 961.6
NDEA	89	125.0	137.2 ± 373.6
NPYR	58	0.5	0.8 ± 2.2
NPIP	60	1.0	2.9 ± 6.1
NMB ₂ A	96	10.0	11.5 ± 29.3
Total	100	406.0	499.9 ± 1313.5

Note. No. of samples was 60; 25 g of the sample with 100 mg NaNO₂ was cultivated at 37°C for 4 h, then extracted and determined by the method of Gas Chromatography-Thermal Energy Analyzer.

DISCUSSION

Esophageal cancer was the second-leading cause of all cancer deaths in China in the 1970s^[10]. The reasons for the exceptionally high risks are not well understood, but dietary factors (such as NOC) together with other factors, have been hypothesized^[11,12]. The present study suggests that the relatively high content of volatile N-nitrosamines is present in the diet of inhabitants from high-risk area of esophageal cancer in Southern China. Its average content was 312 $\mu\text{g}/\text{kg}$ and the daily intake per person estimated was 287 $\mu\text{g}/\text{head}/\text{day}$. The average detectable rate of 5 volatile N-nitrosamines was over 30%. Its total detectable rate was up to 98%. The contents and intakes of NDMA, NDEA and NMB₂A were higher than those of NPYR and NPIP. The second result is the fact that the ability to exogenously synthesize NPIP was powerful in the samples. It suggests that there are conditions for NPIP synthesis in the diet samples. Because NMB₂A and NPIP could specifically induce esophageal cancer in animals, we should pay attention to the phenomenon, because there are high content of NMB₂A and powerful synthesis of NPIP in Nan'ao diet. Correlation of NMB₂A and NPIP with exceptionally high risk of esophageal cancer in this population will be studied in future. As the third result, the total daily intake of nitrite and nitrate was 7.3 mg/head/day. Generally speaking, the more foods contain the amount of precursors of N-nitrosamines, the more foods contain the amount of N-nitrosamines. Sometimes, no such association exists between them. We found that only under the circumstances of the high content of NO \bar{x} , NDEA and NMB₂A, the NDMA content could be raised in the diet. It suggests that the NDMA formation correlates with multiple factors in the diet. Finally, the source of NMB₂A might be fried food. Greengrocery might inhibit formation of NPYR. Altogether, these findings suggest a higher exposure to volatile N-nitrosamines in the inhabitants from high-risk area of esophageal cancer in Southern China. On dietary habit, we advocate that more greengrocery and less fried food should be in foods of three meals. To reduce the amount of carcinogen in foods is one of the important ways for cancer prevention.

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