

A Case-Control Study On Dietary Nutrition and Dietary Habits of Kazakh Esophageal Cancer Patients in Xinjiang

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Abstract

Objective To explore the causes of esophageal cancer (ESCC) and provide a theoretical basis for prevention and treatment of ESCC by analyzing dietary nutrients intake and other blood indicators of Kazakh patients with ESCC.

Methods 198 ESCC cases and 200 controls were recruited in Xinjiang. The food frequency questionnaire was used to investigate the dietary nutrients intake. After collecting blood samples, the automated chemiluminescence immunoassay analyzer was applied to measure serum FA level and serum vitamin B12 level. The enzyme-linked immunosorbent assay was used to observe the serum DNMT1 level.

Results The cholesterol intake of ESCC group was higher than that of the control group while the intakes of protein, thiamin, riboflavin, FA, vitamin A, B6, C and E were lower than the control group. Levels of serum FA and serum vitamin B12 in ESCC group were lower, while the serum DNMT1 level was higher.

Conclusion Lacking fresh vegetables, low educational level, low income, alcoholism, eating solid and dry food and smoked meat, dieting irregularly, low serum FA level, high serum DNMT1 level were risky factors of ESCC for Kazakhs. The occurrence of ESCC reduced with the increase of serum FA but increased when serum DNMT1 increased.

1. Introduction

Esophageal cancer (ESCC) is one of the most common malignant tumors in the world, which seriously threatens people's physical and mental health. About 291000 people are diagnosed with esophageal cancer each year in China, which ranks sixth and fourth place in malignant tumors, with the high incidence rate of 11.28/100,000 and the high mortality rate of 8.36/100,000 reported by the China Cancer Registry(1). The incidence of ESCC varies among different regions, and significant geographical distribution is the prominent epidemiological feature of ESCC(2). Kazakhs who normally inhabit Xinjiang perform the highest incidence of ESCC among all the ethnic groups in China(3), with an adjusted mortality rate of 68.88/100,000. Many Kazakhs depend on stock farming and live in a unique geographical environment and keep long-standing lifestyle and dietary habits, such as smoking, alcoholism, drinking hot milk tea, and eating smoked meat while eating few fresh vegetables and fruits (4).

Folic acid (FA) is a water-soluble B-vitamin that is mainly identified in citrus fruits, green leafy vegetables, beans, grains and animal livers (5-7), which plays an essential role in the maintenance of body's function. Inadequate intake of fruits and vegetables is likely to cause the deficiency of FA. Since it cannot be synthesized by the human body, FA must be obtained from food (mainly from vegetables and fruits) in order to satisfy normal physiological activities through exogenous supply. Evidence shows that the deficiency of FA plays an important role in triggering cancer(8) by two forms of mechanism: one is the erroneous incorporation of uracil into DNA that leads to chromosomal breakage and mutations; the other is the change of expression of key proto-oncogenes and tumor suppressor genes through abnormal

methylation. The main active metabolite of FA is Tetrahydrofolate (THF) that carries one carbon unit to participate in one carbon unit metabolism and methionine cycle in the human body, which acts as a vital coenzyme in reactions to transfer single carbon groups. Lacking Vitamin B12 (VB12), which is a coenzyme synthesized by methionine, can affect methionine synthesis and further undermine DNA methylation and regeneration of THF, eventually causing the relative shortage of FA.

DNA methyltransferases (DNMTs) mainly regulate the process of DNA methylation that is an important form of the base modification in genomes, as well as catalyze the combination between methyl groups and cytosine rings. DNMTs have been identified three major types: DNMT1, DNMT2 and DNMT3, among which DNMT1 is the most predominant one. In details, many studies proved that the abnormally high expression of DNMT1 protein existed in several types of tumor cells (8-10). Furthermore, the high expression of DNMT1 protein and the lack of FA show a positive interaction. The study conducted by Piyathilake et al(11) showed that FA affected the expression of DNMT and the DNA methylation and the gene expression might be improved by supplementing FA, which is able to effectively prohibit the increase of cervical cancer cells and reverse the abnormal transcription and abnormal post-transcriptional expression of DNMT1. Low serum FA level and high expression of DNMT1 protein were also suggested to increase the risks of cervical cancer and precancerous lesions(12-13).

The aim of this study is to provide an experimental basis for prevention and treatment of ESCC in Kazakh populations. Therefore, we carried out a group-matched case-control study in order to understand the relationship among FA and other relevant indicators and ESCC, 398 Kazakh ESCC patients and controls we recruited in Xinjiang from 2010 to 2019 to investigate their dietary habits and structures, as well as the intake of FA and other nutrients. Blood samples were collected to check their serum FA level, vitamin B12, and DNMT1 level and further analyzed the relationship between these factors and ESCC.

2. Materials And Methods

2.1 Subjects

A group-matched case-control design was used in this study according to the principle of same gender, a similar age (within 5 year) and same region. 198 Kazakh patients with ESCC were included as the case group and 200 healthy Kazakhs were collected as the control group in the First Affiliated Hospital of Xinjiang Medical University and Affiliated Cancer Hospital of Xinjiang Medical University between 2010 and 2019. The inclusion criteria of the case group consisted of: subjects who are Kazak ethnic group within 3 generations; lived in the area with the high incidence of ESCC in Xinjiang for more than 5 years; diagnosed ESCC by endoscope, X-ray, and pathology. The control population in this study came from two parts: hospital and population. Hospital: Kazak patients who have no blood relationship but the same region and gender with the cases are selected, the age difference between cases and controls are under 5 years. The patients who have tumor and digestive tract disease at the same time in hospital are excluded. Take out patients with a history of taking vitamins such as folic acid, vitamin B12 and recent infection, fever, autoimmune diseases and active diseases. Population: healthy residents with the same gender and

age difference under 5 years as the case group who have lived in the area with high incidence of esophageal cancer for more than 5 years and have been confirmed free of esophageal cancer through annual local esophageal cancer screening (relying on the screening of early diagnosis and early treatment of esophageal cancer) were selected. Patients with a history of taking vitamins such as folic acid and vitamin B12 were also excluded, and patients with recent infection, fever, autoimmune diseases and active diseases were excluded.

2.2 Esophageal cancer diagnosis

The standard of diagnosis for ESCC was: Barium meal X-ray film of the esophagus shows esophageal stenosis (the wall tube was not smooth, mucosal damage); X-ray computed tomography showed the depth of tumor invasion and the presence or absence of metastatic disease (vertical wall); Stomach endoscopy Microscope/esophagoscopy showed destruction of the esophagus, ulcers and new cauliflower-like structures; cytological examination; histological examination.

2.3 Research Method

2.3.1 questionnaire survey

Self-designed general situation questionnaire was measured to collect participant characteristics, personal history of chronic digestive disease, history of smoking, alcoholism and tea drinking, nutritional status and dietary habits. In accordance with the World Health Organization's recommendations on smoking survey methods, "smoking" in this survey was defined as smoking more than one cigarette per day for more than half a year. "Drinking" was defined as having at least one alcoholic drink per week with an alcohol content of 50 ml or more for more than six months. Furthermore, food frequency questionnaires (FFQs) were used to investigate the daily, weekly, monthly or annual intake frequency of each food and the average amount of each food consumed in the past year. The results of the dietary survey were calculated through nutrition calculator V2.70.

2.3.2 blood sample collection and testing

That 5ml of elbow venous blood was collected from the subjects on an empty stomach in the morning. Laboratory tests determined serum folate, vitamin B12 content, and DNA methylation transferase 1 (DNMT1) level.

In the detection, the glass fiber was first coated with a polymer tetrammonium compound to make the surface of the fiber positively charged, so as to capture the negatively charged reaction complex FA, as well as the reaction complex was adsorbed on the fiber, further the fluorescent marker was connected in parallel. The negatively charged polyanion complex was adsorbed to the surface of the positively charged fiber, the fluorescence intensity change rate was measured finally after a series of steps as well as the content of FA is determined.

Coating the microparticles with vitamin B12 antibody increased the reaction area, improved the sensitivity of the reaction, shortened the reaction time, and improved the specificity of the reaction. Vitamin B12 reacted with the vitamin B12 antibody coated on the microparticles during the test. The reaction solution was transferred to the glass fiber to wash away the unreacted vitamin B12 and unnecessary components. The unreacted second antibody (enzyme-labeled antibody) was washed away after the reaction is repeated, and the substrate MUP was added. MUP was decomposed by alkaline phosphatase to produce methylumbelliferone (Mu). The rate of change in fluorescence that was generated after Mu was irradiated intensity, being measured in which was the content of vitamin B12.

The detection of serum DNMT1 used human serum DNMT1 ELISA kit, and the enzyme-linked immunosorbent assay method was used for determination. The level of DNA methyltransferase 1 (DNMT1) in human serum samples was determined according to the principle of double antibody sandwich method. Strictly followed the steps of the kit, adjusted the blank hole to zero during detection, measured the absorbance value (OD value) of each hole in turn, and the detection wavelength was 450nm. Using the concentration of the standard substance as the abscissa and the OD value as the ordinate, a standard curve was drawn, and the concentration of DNA methyltransferase 1 (DNMT1) in the sample was calculated according to the standard curve.

2.4 Statistical Method and Software Selection

Nutrition calculator V2.70 (based on the "Table of Food Composition in China" 2012 edition as the basic database, and "Chinese Residents Dietary Nutrient Reference Intake (DRIs) 2013 edition as the reference standard) was used for calculation. The average daily intake of various nutrients was obtained, and compared with the reference intake of dietary nutrients of DRIs(14). When the intake was lower than 90% of the reference intake, the intake was insufficient, and when the intake was lower than 30% of the reference intake, the intake was seriously insufficient.

EpiData 3.2 software was used to establish the database and SPSS 17.0 software was applied for analysis. As for univariate analysis, the difference of participant characteristics between two groups was compared by χ^2 test. T test of two independent samples was used for comparison of various types of food intake between two groups. The results of serum folate, vitamin B12, DNMT1 levels between two groups were compared by rank sum test. Multivariate analysis was carried out through unconditional binary logistic regression to identify the risk factors for ESCC.

All included patients gave their oral and written informed consent. This study in which participants gave informed consent was supported by the Medicine Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (20160303-01).

3. Results

3.1 General Situation

As shown in **Table I**, the average ages of the case group and the control group were 58.27 ± 8.01 and 58.43 ± 8.96 respectively. There was no difference between the two groups ($P > 0.05$), except for educational level ($\chi^2 = 41.693$, $P < 0.001$) and income ($\chi^2 = 31.372$, $P < 0.001$).

3.2 Comparison of Dietary Structure

According to the results of FFQ (**Table I**), the intakes of livestock and poultry meat and milk in case group were higher than the recommended amount while the intakes of vegetables, fruits, eggs, and beans were lower than the recommended amount. Both groups seldom ate fish or shrimp. Although the intake of livestock and poultry meat and milk did not differ from each group, the two groups were statistically different in intake of the rest of the foods ($P < 0.05$).

3.3 Comparison of Nutrient Intake

There was no difference in energy intake between the two groups (**Table I**). The cholesterol intake of case group was higher than that of the control group while the intake of protein, vitamin A, thiamine, riboflavin, vitamin B6, FA, vitamin C, and vitamin E was lower than that of the control group and the difference was significant ($P < 0.05$). According to the DRIs(3) recommended by the Chinese Nutrition Society for Chinese residents, the intake of thiamine, vitamin B6, vitamin E, and FA in both groups failed to reach 90% of the reference intake, and the FA intake of the case group did not reach 30% of the reference intake, which was severely inadequate.

3.4 Comparison of Serum Folic Acid, Vitamin B12 and DNMT1 Levels

Levels of serum FA and vitamin B12 in case group were significantly lower than those of the control group (**Table I**) ($z = -9.13$, $P < 0.001$; $z = -6.06$, $P < 0.001$), whereas the serum DNMT1 level in case group was higher than that of the control group, and there was statistical significance ($z = -5.37$, $P < 0.001$).

3.5 Logistic Regression Analysis of Risk Factors for Esophageal Cancer in Kazakhs

Multivariate binary logistic regression model was performed to analyze the influence of variables including education level, income, smoking, alcoholism, taste preference, vegetable and fruit intake, eating solid and hard food, eating quickly, dieting on time, eating smoked meat, serum folate level, serum vitamin B12 level and serum DNMT1 level (**Table I**). The result showed that eating smoked meat was primary risk factor for ESCC (OR = 12.812, 95%CI: 4.793-34.248), followed by serum DNMT1 level, alcoholism and eating solid and dry food ($P < 0.05$). By contrast, serum folate level was identified as the primary protective factor for ESCC (OR = 0.021, 95%CI: 0.004-0.101). Dieting on time and vegetable intake also played an important role in protective in ESCC happening ($P < 0.05$). Notably, the result demonstrated education level and income influenced the presence of ESCC as well ($P < 0.05$).

3.6 Relationship among Serum Folic Acid Level, DNMT1 level and Esophageal Cancer

Since the serum FA and serum DNMT1 levels in the case group were significantly different from those in the control group ($P < 0.001$), we further analyzed their relationships with ESCC. We divided serum FA level and DNMT1 level in the control group into four categories based on their quartiles (**Table 1**). The risk of getting ESCC in the second, third and fourth subgroup were 0.350, 0.167 and 0.150 times of that in the first subgroup respectively ($\chi^2 = 15.281, 32.650, 34.642, P < 0.001$).

As shown in **Table 1**, on the contrary, the risk of getting ESCC increased with serum DNMT1 level, though such tendency was only significant with serum DNMT1 level above 9.77ng/ml. In particular, the group with highest DNMT1 level above 9.77 underwent 4 times of the risk of ESCC compared to the group with lowest DNMT1 level ($\chi^2 = 21.353, P < 0.001$) but there was no significant relationships between ESCC and the group with serum DNMT1 level 6.39~7.67ng / ml or the group with serum DNMT1 level 7.68 ~ 9.77ng / ml.

4 Discussion

Many recent studies(15-18) have demonstrated that ESCC is related to various genes and develops through several stages and the deficiency of FA or FA metabolism disorder may cause the cancer by disrupting the normal methylation and the synthesis of DNA. Most of the Kazakhs lead a nomadic life, with a low level of education, poor living standards and a monotonous diet that can easily result in the insufficient intake of nutrients. The possibility of suffering from ESCC increases with the effect of these factors.

The current study found that Kazakhs consumed more animal-based food and less fresh vegetables and fruits, which led to higher protein and cholesterol intakes and lower vitamin intake than DRIs. The case group had inadequate intakes of thiamine, vitamin E and vitamin B6, and the intake of FA only reached 28.88% of DRIs. Eating few vegetables and fruits can easily cause the deficiency of FA mainly existing in fresh vegetables(5,19). Taking more fresh vegetables, milk and eggs can effectively prevent ESCC.

A burden on the digestive system can be the result of unhealthy living and dieting habits, furthermore, which stimulate the esophagus. Several epidemiological studies supported that people with a higher risk of ESCC have the following dietary habits: preference of hot food, excessive intake of pickled food, lack of fresh fruits and vegetables, and regular intake of dry and solid food(20-22), which is basically consistent with the results of our multivariate analysis. The preference of beef and mutton is mainly attributed to the pattern of herding. They cannot eat on time result of they have to herd and often keep dry and solid food such as dried Naan and dried cheese as staples. They often eat smoked food like smoked horse meat and smoked sausages during the long and cold winter while eating less vegetables and fruits. Notably, the subjects of the current are all Kazakhs who generally enjoy hot food, which showed no significant difference between two groups and was different from previous studies.

The current study showed the serum FA of case group was significant lower than the control group. The risk of ESCC would be higher with the serum FA level is lower. For example, Bao Liuli et al. applied enzyme-linked immunosorbent assay (ELISA) to measure the serum FA level of three groups and showed

that serum FA levels in the ESCC group were lower than that in the precancerous lesion group and control group, indicating that the occurrence and development of ESCC are related to the deficiency of FA(23). Similarly, Hao Ting et al. confirmed that the risk of getting ESCC increased when the serum FA level was low in their study concerning the relationship between Kazakhs' serum FA level and ESCC(24). The chance of high serum FA group to suffer from ESCC was 0.16 times of that of the lowest serum FA group, showing that serum FA level was negatively correlated with the risk of ESCC. The relationship between serum FA level and ESCC in this study is consistent with that between dietary folate intake and ESCC, suggesting that there may be a correlation between dietary folate and serum FA.

DNA methylation refers to a mechanism that affects and regulates gene activity, in which DNMTs plays an important role(25-26). In details, study suggested that DNMTs expressed abnormally in many diseases. Notably, DNMT1 is the focus of DNA methylation research and the expression significantly increased in some malignant tumors such as gastric cancer, colon cancer, breast cancer, prostate cancer, bladder, cervix, lung, esophagus, brain, pancreas(27-32). For instance, several studies confirmed that the abnormal expression and activity of DNMTs would lead to the abnormal hypermethylation of certain oncogenes in tumor cells(33-35). The occurrence of tumors could be accounted by DNA methylation, which also exists in precancerous lesions and early stages of cancerization. In particular, abnormal expression of DNMT1 was related to the occurrence of ESCC and it may be involved in the development of ESCC(36). The current study also showed that the serum DNMT1 level was higher than that in the control group, which suggested that the higher DNMT1 level the higher possibility of getting esophageal cancer. Therefore, it is likely to be an optimal marker for early diagnosis of tumors. Changing the status of DNA methylation may provide new solutions for cancer prevention and treatment(37). However, we still need further studies to explore the exact mechanism of DNMT1 involved in ESCC.

The FFQ we used to investigate the dietary nutritional status of subject may exist memory bias. Because some nutrients (such as vitamin B12) have no measured values in most foods in China. The results of the intake of dietary nutrition may have certain limitations, which can be improved in future studies. Although mechanism of ESCC has not been fully clarified, based on current epidemiological studies, we can at least provide some preventive strategies to Kazakhs, such as enhancing health education and raising awareness of prevention of ESCC, doing regular health examination, changing unhealthy dietary habits including preference of dry, hard and hot food and quick-eating, eating more fresh vegetables, fruits and bean products.

Declarations

Ethics approval and consent to participate: All included patients gave their oral and written informed consent. This study in which participants gave informed consent was supported by the Medicine Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (20160303-01).

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Competing Interests The authors have declared that no conflict of interest exists.

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Author Contributions: The integrity of the entire study was guaranteed by Yan CHEN, as well as performed the manuscript revision/review. Dong YIN performed study concepts, study design and experimental studies. Yu YIN performed experimental studies, data acquisition and writing-original draft preparation. Siyao Li performed literature research, experimental studies and data acquisition. Xufeng LI performed data acquisition, data interpretation. Yanchao DENG performed data acquisition.

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Tables

Table □ General situation of case group and control group

Factor		Sum	Case group [n %]	Control group [n %]	χ^2	P
Gender	Male	272	130 (65.66%)	142 (71.00%)	1.568	0.234
	Female	126	68 (34.34%)	58 (29.00%)		
Age	≤50	81	36 (18.20%)	45 (22.50%)	3.743	0.291
	50~	157	80 (40.40%)	77 (38.50%)		
	60~	128	70 (35.34%)	58 (29.00%)		
	≥70	32	12 (6.06%)	20 (10.00%)		
Education	Below secondary	194	129 (65.15%)	65 (32.50%)	41.693	≤0.001
	Secondary and above	204	69 (34.85%)	135 (67.50%)		
Marital Status	Married	361	183 (92.42%)	178 (89.00%)	-	0.092*
	Widowed	31	14 (7.07%)	17 (8.50%)		
	Divorced	5	0 (0.00%)	5 (2.50%)		
	Unmarried	1	1 (0.51%)	0 (0.00%)		
Income	≤1000	292	143 (72.22%)	89 (44.50%)	31.372	≤0.001
	>1000	166	55 (27.78%)	111 (55.50%)		
	Sum	398	198 (100.00%)	200 (100.00%)		

Table 1 Comparison of various types of food intake between case group and control group

Food Type	Recommended Intake(g)	Actual Intake(g)		<i>t</i>	<i>P</i>
		Case Group [$\bar{x} \pm s$]	Control Group [$\bar{x} \pm s$]		
Grains, Potatoes	250-400	316.76±58.25	383.47±60.31	11.124	≤0.001
Vegetables	300-500	228.06±47.17	292.73±52.87	12.761	≤0.001
Fruits	200-400	102.79±58.83	191.82±53.63	14.262	≤0.001
Poultry, Livestock Meat	50-75	311.90±98.70	317.03±94.52	0.526	0.599
Fish, Shrimp	50-100	0	0	-	-
Eggs	25-50	11.03±11.45	21.76±15.36	6.362	≤0.001
Milk	300	323.36±180.52	350.72±195.72	1.962	0.051
Beans	30-50	9.04±7.31	13.70±6.89	2.676	0.008

Table 1 Comparison of nutrient intakes between case group and control group

Nutrient	DRIs	Actual Intake			
		Case Group $\bar{x} \pm s$	Ratio with DRIs	Control Group $\bar{x} \pm s$	Ratio with DRIs
Energy (Kcal)	2100	1922.28±306.26	91.54	1983.07±319.26	94.43
Protein (g)	65	84.91±20.00*	130.63	103.94±21.04	159.91
Cholesterol (mg)	300	455.90±145.43*	151.97	362.35±133.81	120.78
Vitamin A(μgRE)	800	847.42±229.70*	105.92	1178.00±263.85	147.25
Thiamine (mg)	1.4	0.73±0.14*	52.14	1.23±0.19	87.86
Riboflavin (mg)	1.4	1.32±0.30*	94.28	1.50±0.33	107.14
Vitamin B ₆ (mg)	1.6	0.63±0.15*	39.37	1.05±0.17	65.62
Folic Acid (μg)	400	112.88±13.54*	28.88	225.84±15.05	56.46
Vitamin C(mg)	100	99.61±25.88*	99.61	120.29±25.20	120.29
Vitamin E(mg)	14	8.04±2.26*	57.42	10.55±2.23	75.35

Table 1 Comparison of serum folate, vitamin B₁₂, DNMT1 levels between case group and control group

Tested Indicators	Case Group $\bar{x} \pm s$	Control Group $\bar{x} \pm s$	Z	P
Serum Folic Acid (nmol/L)	6.42±2.69	9.04±4.13	-9.13	≤0.001
Serum Vitamin B ₁₂ (pmol/L)	236.36±90.67	302.00±137.46	-6.06	≤0.001
Serum DNMT1 (ng/ml)	11.15±8.60	8.55±4.34	-5.37	≤0.001

Table 2 Multivariate logistic regression analysis of esophageal cancer

Variables	<i>B</i>	<i>SE</i>	Wald χ^2	<i>P</i>	OR(95% CI)
Education Level	-1.898	0.418	20.658	≤ 0.001	0.150 [0.066~0.340]
Income	-1.569	0.434	13.052	≤ 0.001	0.208 [0.089~0.488]
Alcoholism	0.982	0.446	4.844	0.028	2.670 [1.113~6.402]
Vegetable Intake	-1.711	0.420	16.631	≤ 0.001	0.181 [0.079~0.411]
Eating Solid and Dry Food	0.827	0.401	4.246	0.039	2.287 [1.041~5.022]
Dieting on Time	-3.369	0.785	18.404	≤ 0.001	0.034 [0.007~0.160]
Frequently Eating Smoked Meat	2.550	0.502	25.845	≤ 0.001	12.812 [4.793~34.248]
Serum Folate Level	-3.886	0.812	22.874	≤ 0.001	0.021 [0.004~0.101]
Serum DNMT1 Level	1.160	0.494	5.509	0.019	3.191 [1.211~8.411]

Table 1 Analysis of the relationship between different levels of serum folic acid and the risk of getting esophageal cancer

Serum Folic Acid [nmol/L]	Case Group [n=198]	Control Group [n=200]	Wald χ^2	<i>P</i>	<i>OR</i>	<i>95%CI</i>
≤ 6.26	117	50			1.000	
6.27~8.12	41	50	15.281	0.000	0.350	0.207~0.593
8.13~10.20	21	50	32.650	0.000	0.167	0.090~0.308
≥ 10.20	19	50	34.642	0.000	0.150	0.080~0.282

Table 2 Analysis of the relationship between different levels of serum DNMT1 and the risk of getting esophageal cancer

Serum DNMT1 Level (ng/ml)	Case Group [n=198]	Control Group [n=200]	Wald χ^2	<i>P</i>	<i>OR</i>	<i>95%CI</i>
≤ 6.38	26	50			1.000	
6.39~7.67	29	50	0.111	0.739	1.120	0.575~2.182
7.68~9.77	40	50	1.872	0.171	1.560	0.825~2.950
≥ 9.77	103	50	21.353	0.000	4.000	2.222~7.201