Comparative research for the dietary pattern of patients with esophageal cancer at different developing stages and the daily intake of vitamin A, E and β-carotene

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Abstract: This paper discusses the different stages of normal esophageal's developing to esophageal cancer, and the difference among dietary patterns of patients with esophageal cancer and acceptable daily intake of vitamin A, E and beta carotene intake in diet. This paper takes advantage of food composition table, calculates the intake amount of dietary vitamin A, E and beta carotene in all kinds of food for patients with esophageal cancer, and analyzes the intake amount difference of dietary vitamin A, E and beta carotene in each kind of food for different groups of people. Research conclusions: the low content level of dietary vitamin A, E beta-carotene and low intake amount of beans, vegetables and fruit intake may increase the risk of esophageal cancer' occurring, while the relationship among dietary vitamin E, the occurrence and development of esophageal cancer needs further discussion.

Keywords: Patients with esophageal cancer, Vitamin A, E, Beta-carotene.

INTRODUCTOIN

Esophageal cancer ranks the eighth in the incidence of malignant tumors in the world, for male, it is twice as many as female. China is the country, which has the highest morbidity and mortality of esophagus cancer in the world, and Chinese cities rank the fourth in the cause sequence of the malignant tumor death in the world, the rural ranks the third (Wenxian *et al.,* 2008). The mortality of esophageal cancer shows a downward trend in recent years, but still at a higher level. The occurrence of esophageal cancer depends largely on environmental factors, in which the dietary patterns and nutritional status is in important position, but the research about the relationship between vitamin A, E, beta carotene and esophagus cancer is inconsistent. In this paper, we perform the comparative analysis for the dietary patterns and vitamin A, E, beta-carotene intake of the patients with esophageal cancer in different development stages. The function of resisting esophagus cancer of vitamin is getting more and more attention of people, many data indicate that vitamin A, E and beta carotene is associated with the occurrence of cancer (Qingtao *et al.,* 2010). This paper mainly selects patients with esophageal cancer in some hospitals of Hejian village in Linzhou city as the research objects, so as to understand the relationship between Vitamin A, E, beta-carotene and cancer (Yanlin *et al.,* 2010).

Recently there have been many reports about the life style and esophagus cancer, which are related with many drinking and eating habits like smoking and drinking etc. Smoking is an already decided known cause of esophagus cancer, the diet composed of vegetable and fruit would reduce the risk of esophagus cancer. And there is sufficient evidence demonstrating that alcohol drinking would increase the risk of esophagus cancer.But as the relationship of life-style and study area is extermely close, the research in different area always give different conclusion. Through the research about comparing disease case with epidemiology, this paper has discussed the relationship between dietary patterns, vitamin A, vitamin E,β-carotene and esophagus cancer. It has made positive contribution to providing scientific basis for obtaining the early prevention and treatment of nutritional indicators about esophagus cancer, reducing the morbidity and mortality of esophagus cancer, improving the nutritional status of crowds.

METHODS

Research object: Extract four villages in HeJian township of Linzhou city through using simple random sampling method, according to the results of the census and pathology inspection, it screens the objects with complete dietary information and no other diseases of the digestive system, it ultimately collects 173 cases of normal residents (normal group), 281 cases of mild dysplasia (light increase group), 50 cases of moderate dysplasia (moderate increase group), 29 cases of severe dysplasia (severe increase group), 30 cases of patients with esophageal cancer (cancer group), there is a total of 563 cases, among which 301cases are male and 262 cases are female (Wiseman, 2008).

Survey project and content, general situation: Age, gender, household income, educational level and marital status, etc. Dietary intake condition: food type, intaking

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frequency and consumption amount each time. Data collection: through using food frequency questionnaire (FFQ), investigators who have gone through unified training perform survey to all residents who are aged from 40 to 69. The total 605 questionnaires were issued, each completed questionnaire was checked by the responsible officer, and they were taken in strict quality control, finally 563 valid questionnaires (93%) were collected (Carlson and Bultz, 2004). Statistical processing: the survey data was in unified serial number and entered through using Epidata 3.0 (Wang *et al.,* 2005). Collate and assign each index through using FoxPro 6.0, calculate the intake amount of each type of food and vitamins A, E, beta carotene for each people in each group according to the "China food composition table in 2004". Make analysis through adopting SPSS 12.0, inspect and compare the difference of residents' basic situation through using X^2 and compare the difference between the intake of average daily dietary and vitamin A, E, beta-carotene by adopting one-way analysis of variance, and the inspection level α=0.05.

Research results: Comparing the basic situation of residents in each group table 1.

The comparison of dietary type and quantity is shown in table 2. We could know the intake of cereal grains is higher than the recommended dietary intake: the rest kinds of food intake are below the recommended intake. The comparison for the intake of dietary vitamin A, E, beta-carotene among residents in each group is shown in table 2.

RESEARCH DISCUSSION

Numerous studies have proved that dietary nutrition is closely correlated with the esophageal cancer. The author's results show that, in areas with high incidence of esophageal cancer, residents' food is monotonous, grain cereal intake is too much, animal foods, soy foods and fresh fruits, vegetables are in shortage, supply rate of three major nutrients are imbalanced. Unreasonable dietary structure and the intake of nutrients is imbalanced that could be one of the important factors of high incidence of esophageal cancer. The study finds that the vitamin A intake of the residents in areas with high incidence of esophagus cancer is lower than that of residents in areas with low incidence of esophagus cancer, Ping Xiao found the dietary beta -carotene intake in the case group of esophagus cancer were lower than that of control group (Bollschweiler *et al.,* 2002). The authors found that: the intake of vitamin A and beta carotene in normal group was higher than light increase group, the moderate increase group, heavy increase group and cancer group, which suggests that the low content level of vitamin A, beta-carotene in diet may be the risk factor for causing esophageal cancer. Vitamin E has gotten much more attention for its unique anti-oxidation function in research of resisting cancer through diet. But only very limited evidence has suggested that vitamin E is associated with esophageal cancer: Ping Xiao found that the intake of dietary vitamin E and serum vitamin E in case group were significantly lower than the control group. A case-control study abroad suggested that dietary vitamin E may be protective factors of esophagus cancer. The study results show that the average vitamin E intake of residents in Linzhou city is more than 20mg/d, which is higher than the recommended adult 's intake of vitamin E (14mg) and it indirectly reflects the improvement of the dietary nutrition level in recent years, but does not find there existing differences between the dietary intake of vitamin E among local groups of residents in LinZhou, which may be related to that vitamin E intake is generally on the high side (Aggarwal *et al.,* 2010). Relationship between vitamin E and esophageal cancer remains to be further discussed (Rovin and Winn, 2005).

The research experiment analysis

Dietary factors have played an important role in the occurrence and development of esophageal cancer, the residents in HeJian township of Linzhou city where the incidence of esophagus cancer is high have unique eating habits, the relationship between diet habit of the residents in HeJian township of LinZhou city and the high incidence of local esophageal cancer is unclear. Rats is better used as the good animal models for studying nutriology, most research that focus on the relationship between nutrition and disease is to remove or add various nutrient based on the conventional feed of rats, thus explore the relationship between them. There is no person completely simulating adult diet mode on feeding rats, by which to explore the role of diet in cancer development. Existing diet and esophageal cancer research is limited to epidemiological investigation, induced cancer experiment of animal or cell biology research of single nutrients and esophagus cancer (Stogios *et al.,* 2007). This paper simulates the mode of feeding rats with adult diet in view of Chinese medicine serum pharmacology method, exerts the action of serum on esophageal cancer cells through the biotransformation within rats' body, thus avoid the disadvantages of the several traditional methods when used alone, provides new technical platform for further comprehensively discussing relationship between diet and esophageal cancer through using modern biology technology. This lab has already established serum physiology method, and performed pilot study for the relationship among medicine plant extracts, lung cancer, dietary fat and lung cancer, this paper establishes serum physiological approaches of studying the diet and esophageal cancer on this basis. First of all, it sets up the diet control group through simulating the mode of feeding rats with adult diet in animal experiment, observe the growth of rats and explore the feasibility of feeding rats with adult diet, thus make the results of further simulating

the patients' diet in HeJian township of Linzhou city where the incidence of esophagus cancer is high on feeding rats more convincing (Pomerantz *et al.,* 1998). Also this paper sets up the calf serum contrast and normal cells contrast in cell experiment, thus improves the serum physiology method, and makes the experiment results more reliable (Berkovich *et al.,* 2003).

MATERIALS AND METHODS

Material

Feed preparation

(1) Feed 1: rats' conventional feed is purchased from laboratory animal center of Sichuan University.

(2) Feed 2: prepare rat feed through simulating adult diet in Chengdu, food variety and quantity is similar to the national dietary survey of urban and rural residents in 2002, the food is divided into four categories: valley potato; fruits and vegetables; poultry, fish, eggs, milk, spices and oil, raw material is purchased from free market in Chengdu. Grain is broken into powder, rubbing them with meat fruits, vegetables after washing and drying them, then they are processed into rats' feed.

Experimental animals

Male rats of SD clean level: the weight is 1709-1909, age is 6-7 yearling, they are purchased from laboratory animal center in Sichuan university, qualified animal certificate No: SCXK (Sichuan) 2005 - 09.

Cell plant

Eca-109 is cell plant of squamous carcinoma on human esophageal epithelium, they are purchased from the Shanghai institute of cell biology in Chinese academy of sciences; the normal cell plant HL7702 of human liver epithelial is provided by immunization sanitarium of Huaxi basic medicine and forensic sciences in Sichuan university (because China has no normal cell plants of esophageal epithelium, so this study selects the normal cell plants of liver epithelial whose origin is human epithelium as normal cell contrast).

20 SD rats were randomly divided into two groups by weight: DS group: control group of normal rats feed; CD group: adults' diet group in Chengdu (Chengdu diet group in abbreviation). 2 groups of rats eat feed and drink plain boiled water freely. Record feed intake timely each day, record weight weekly, feed them 30 days. Animal are fed in breeding rooms of barrier facilities in the center of West China school of public health, its temperature is about 2 □, relative humidity is 50-60%. Alternative time of day and night time is 10-14h.Add feed and replace the water, replace the bedding three times a week, clean disinfection cage and water bottle three times every week, clean and disinfect breeding rooms twice.

Grouping and processing cell experiment

Regular training of cell

Through using RPMI1640 nutrient solution, which contains 10% calf serum (56^{o}C inactivated 30min), buffer pH to 7.2. The cell adopts open single-layer adherent culture in 37^{o} saturated humidity and 5% CO condition, which are put in CO incubator for regular training. Two plant cells of Eea-109, HL7702 are all adherence growth cell, and they are developed in the same way.

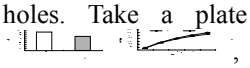
Serum preparation

Feed rats with conventional feed for 30 d, extract femoral artery blood, put it in stewing at 37^{o} for 2 h, centrifuge it for 10 min at 3000 RPM, absorbs the serum, perform aseptic filtration packing to the same group of serum after blending them, the hemolysis is abandoned, and preserve it in 20□ for later use.

The grouping of cell experiment

Perform conditional screening experiment to rats serum (blank serum in rat) which are fed with conventional feed, in order to determine the optimum concentration of serum which is added into nutrient solution and appropriate treatment method. Experimental grouping are as follows: ①-④group: perform cell culture to the nutrient solution which contains 2.5%, 5%, 10%, 15% non inactivated serum in rats; ⑤-⑧ group: perform cell culture to the nutrient solution which contains 2.5%, 5%, 10%, 15% non inactivated serum in rats; ⑨let the nutrient solution which contains 10% inactivation serum be control group (XN group).

Cell growth curves drawn by MTT method

The testers extract Eca-109 cell of logarithmic phase, digest and blow digestive juice (0.25% pancreatic enzymes+0.01%EDTA) into single-cell suspension, perform microscopic count after 0.4% Trypan blue staining. Adjust the cell concentration with the nutrient solution which contains 10% calf serum to be 1.oxlo4/ml, perform vaccination in 96 - well plates with 100 Sichuan/hole, cultivate it in CO cultivate; discard nutrient solution after 24h, add nutrient solution which contains different groups of rat serum, let each group have four holes. Take a plate for each respectively on the  , add MTT (1mg/ml) 50Lin l/hole, discard nutrient solution after cultivating 4h, add DMSO150 Sichuan/hole, perform percussion and blending, fully dissolve purple crystal saving, measure and absorb luminosity A at 490 nm wavelength on microplate reader. Testers take incubation time as abscissa, regard absorbancy A as vertical coordinates, draw growth curve of cell.

The influence of blank serum in rats on normal liver epithelial cells

We know from the results of MTT method that 5% inactivated serum of rats is the most suitable for Eca-109

Table 1: The comparison for the basic situation of people in each group

Group	N (male/female)	40～year	50～year	59～69 year
Normal group	173 (86/87)	54	94	25
Slight increase group	281 (148/133)	99	126	56
Moderate increase group	50 (29/21)	16	23	11
Heavy increase group	29 (16/13)	8	15	6
Cancer group	30 (18/12)	10	13	7
X^2	1. 896		5. 736	
P	0. 755		0. 677	

Table 2: The comparison for the average daily dietary intake among residents in each group

Group	n	Cereal grains	Meat, eggs, fish and shrimp	Dairy products	Beans	Greengrocery	Fruit	Salt
Normal group	173	581.61±216.94	50.76 ±45.49	29.76±65.87	40.94±66.15	253.39±101.56	39.37±43.63	0.00±0.02
Slight increase group	281	567.94±221.95	40.71 ±44.43	28.47±53.11	28.01±48.64*	227.22±91.33*	27.85±44.26*	0.01± 0.04
Moderate increase group	50	550.50±212.67	34.71±26.85	43.74±71.77	22.53±47.96*	208.22±84.89*	18.58±19.21*	0.01±0.06
Heavy increase group	29	640.05±258.89	41.08±25.75	27.09±55.72	25.65±25.49	196.60± 95.01*	33.57±37.69	0.01±0.01
Cancer group	30	630.70±269.72	40.92±23.89	33.96±73.66	20.96±22.10	238.05±101.66	29.92±46.33	0.02±0.06
F		1.308	2.211	0.735	2.417	4.203	3.198	0.649
P		0.266	0.067	0.568	0.048	0.002	0.013	0.628

*Compared with normal group, P<0.05.

Table 3: The comparison for the average daily intake of vitamin A, E, beta-carotene among each groups of residents

Group	N	Vitamin A	Vitamin E	Carotene
Normal group	173	180.88 ±120.92	25.48 ±12.16	350.09 ±160.19
Slight increase group	281	148.35 ±111.04*	24.13 ± 12.66	301.10 ± 139.98*
Moderate increase group	50	140.13 ± 75.41*	23.49 ± 12. 82	277.42 ± 115.61*
Heavy increase group	29	137.34 ± 82.07*	22.02 ± 9.96	268.45 ± 122.26*
Cancer group	30	154.10 ± 60.45	20.50 ±11.17	302.38 ±137.83
F		3.099	1.417	4.863
P		0.015	0.227	0.001

*Compared with normal group, P<0.05.

growth proliferation of esophageal carcinoma cell, in order to observe whether rat serum is suitable for growth and proliferation of normal cells. This experiment replaces calf serum with 5% inactivated serum of rats, in order to cultivate epithelial cells of normal liver in human which is HL7702, in reference to the method in MTT method, draw growth curve of cell with MTT method.

STATISTICAL ANALYSIS

Denote feed intake, weight and absorbancy A value in different groups of rats with Castel, perform variance analysis for the t inspection of two sample mean comparison and diverse mean comparison through using SPSSI 3.0 statistical analysis software, which makes P<0.05 be difference and have statistical significance.

RESULT

The influence of feeding rats with Chengdu diet on its growth The comparison for the composition between formula feed diet in Chengdu and conventional rats' feed, as well as its essential nutrient, it is shown in the table 4 The influence of Chengdu diet on the average rats' intake and weight, it is shown in the fig. 1 The feasibility of substituting calf serum with rats' serum in cultivating cells The influence of rats' serum on growth proliferation of human esophageal cancer cell under different conditions

We could see from fig. 2 (A), non inactivated serum in rats cultivates esophageal carcinoma cell Eca-109 under different concentration conditions, the cell basically represents the trend of index increase, which is approaching to the cell growth trend in control group of calf serum. Fig. 2 (B) indicates only as inactivated serum in rats under 5% concentration, Eca-109 cell represents the trend of index increase, which is approaching control group of calf serum, while under the other three concentration conditions, all the cells grow slowly. Under the condition of rat serum concentration is 5%, all the Eca-109 cells represent the trend of index increase, which

is approaching to the cell growth trend of calf serum in the control group, cell's proliferation ability is higher than the other three concentration groups.

Fig. 2 (A), (B) show the influence of each group of serum on growth and proliferation of Eca-109 cell is different, 72h difference is more obvious ($F=159263$, $P<0.05$). The results of pairwise comparison indicates that, the influence of 5% non-inactivated serum in rats on cell growth and proliferation is close to the control group of calf serum ($P>0.05$), while the growth proliferation ability of cells in this group is higher than other groups, and there is statistical difference ($P<0.05$). This suggests that 5% non-inactivated serum in rats is the most suitable for the growth of Eca-109 cell, so they select the rat serum in such condition to do further research (Suzuki *et al.,* 2005).

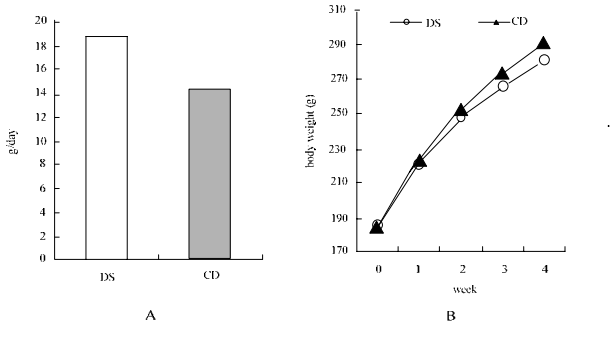


Fig. 1: The influence of Chengdu diet on rats' average feed intake (A) and body weight increase (B) The feasibility of substituting calf serum with rats ' serum in cultivating cells the influence of rats' serum on growth proliferation of human esophageal cancer cell under different conditions

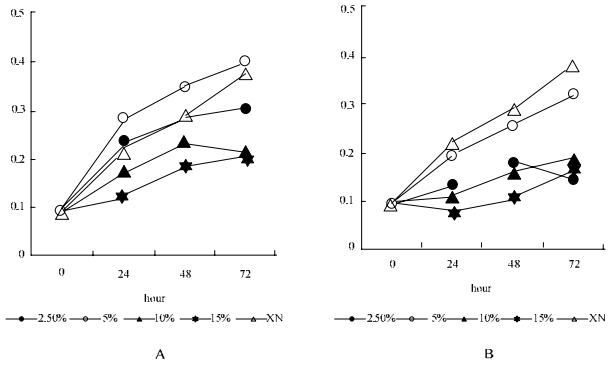


Fig. 2: The influence of rats' non-inactivated serum (A) and inactivated serum (B) on the growth curve of esophageal cancer cells (ca-109).

DISCUSSION

This experiment performs simulation of rats' feeding with adult diet in Chengdu, its weight increase has no obvious difference with rats' group of conventional feed, which suggests that adult diet in Chengdu has no obvious influence on rats, which suggests that feeding rats with adult feed is feasible. This experiment cultivates esophageal carcinoma cell of human being through substituting traditional calf serum with blank serum in rats which is inactivated or non inactivated respectively with different concentration, and it screens out that 5% non inactivated blank serum in rats is the most suitable for rats' growth. The influence of 5% non-inactivated blank serum in rats on cell growth of normal liver epithelial is close with the effect of calf serum, and it suggests such serum is equally suitable for the normal cell growth. This experiment establishes and perfects the serum physiology method through constructing the animal model of simulating adult diet, and setting up the contrast of calf serum and normal cells respectively, thus provides new technology for discussing the relationship among diet, nutrition and esophagus cancer (Agrawal *et al.,* 2006).

Table 4: The influence of Chengdu diet on rats' average feed intake and body weight (g)

Group	Diet intake	Body weight
DS	18.57±0.33	254.0±25.81
CD	14.43±0.19[a]	260.75±28.79

CONCLUSION

This experiment result indicates that, the effect of vitamin combination 2 (vitamin A, E, folic acid, riboflavin, vitamin C) is stronger than vitamin combination 1 (vitamin A, E, folic acid), but they have no obvious difference. It suggests that riboflavin and vitamin C may strengthen the effect of vitamin A, E, folic acid in regulating cell cycle. Whether these kinds of vitamin have synergistic effect in regulating cell cycle is waiting for further study. In conclusion, experimental results show that feeding rats' serum with Chengdu diet has promoted the growth and proliferation of esophagus cancer, while feeding the rats serum with the diet added with several kinds of vitamins has inversed the effect of feeding rats with Chengdu diet, its effect mechanism is related with regulating cell cycle (Choi *et al.,* 2009).

FUND PROGRAM

Technological innovation outstanding talents project of H enan province. Project name: Research of Smo SiRNA to Smo and Glil expressive influence in human esophageal cancer cells.

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