# Relationship Between Microcystin in Drinking Water and Colorectal Cancer[1]

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**Objective** To investigate the association of microcystin (MC) in drinking water with the incidence of colorectal cancer. **Methods** The study was designed as a retrospective cohort. Eight townships or towns were randomly selected as the study sites in Haining City of Zhejiang Province, China. 408 cases of colon and rectum carcinomas diagnosed from 1977 to 1996 in the study sites were included, and a survey on types of drinking water of these patients was conducted. Samples of different water sources (well, tap, river and pond) were collected separately and microcystin concentrations were determined by indirect competitive ELISA method. **Results** The incidence rate of colorectal cancer was significantly higher in population who drank river and pond water than those who drank well and tap water. Compared to well water, the relative risk (RR) for colorectal cancer was 1.88 (tap), 7.94 (river) and 7.70 (pond) respectively. The positive rate (>50 pg/mL) of microcystin in samples of well, tap, river and pond water was 0, 0, 36.23% and 17.14% respectively. The concentration of microcystin in river and pond water was significantly higher than that in well and tap water ($P$<0.01). Spearman rank correlation analysis showed that in the study sites, the microcystin concentration of river and pond water was positively associated with the incidence of colorectal cancer ($r_s$= 0.881, $P$<0.01). **Conclusions** The types of drinking water are positively associated with the incidence of colorectal cancer in the study sites, and this may be related to microcystin contamination of drinking water. Further biological study is needed to support the possible causative role of mycrocystin in carcinogenesis of colon and rectum.

**Key word:** Drinking water; Microcystin; Colorectal cancer

## INTRODUCTION

Colorectal cancer is one of the leading causes of cancer death in Western countries. China used to be a low-prevalence area for colorectal cancer, its mortality ranked fifth for men and sixth for women in 1970's, however, its incidence rate has been rapidly increasing for the last 3 decades in the country. The generally accepted causes for colorectal cancer are related to dietary factors, sedentary life style, inheritable factors and other factors[1]. Epidemiological studies in Zhejiang Province, China, revealed that its incidence is closely related to the types of drinking water, the areas where people use surface water (river and

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pond) have higher incidence rate and the risk increases with the year of drinking water[2,3]. Surface water is a highly polluted water source, what contaminants in the water are responsible for colorectal cancer remain unknown.

Blue-green alga (cyanobacteria) are the most common blooms found in eutrophicated surface water, such as *Microcystis aeruginosa*. The released algal toxin (microcystin, MC) has been recognized as the threat to human health, particularly its hepatotoxic effect. Yu *et al*. first reported the association of microcystin with hepatocellular carcinoma[4]. Since intestinal mucosa is directly exposed to the toxin, it is suggested that mycrocystin may be also responsible for the development of colorectal cancer. In order to investigate its possible role in the development of colorectal cancer, a retrospective cohort study was conducted to assess the relative risk of various drinking water sources, to measure the microcystin concentration in different water sources, and to analyze the relationship between the incidence of colorectal cancer and the toxin concentration.

## MATERIALS AND METHODS

### *Subjects*

The study was designed as a retrospective cohort investigation. Eight townships or towns were randomly selected as the study sites in Haining City of Zhejiang Province, China. A total of 408 cases of primary colorectal adenocarcinoma (163 colons and 245 rectums) diagnosed from 1977 to 1996 were included. All cases were obtained from the Cancer Registry of Haining Cancer Research Institute. The pathological sections of all cases were re-examinated by two pathologists independently and the definite diagnosis of colon and rectal cancer was made according to the National Standard for Colo-rectal Cancer Diagnosis[5]. Of these cases 224 were males (54.9%), 184 were females (45.1%). The data on types of drinking water were collected at home interview with the patients (for living cases) or family members (for dead cases) by specially trained interviewers. The types of drinking water were defined as the water source that the patients used for life long or the longest in their lifetime. The data of residents in the study sites and their drinking water sources were provided by the local household registration and relevant sanitation and anti-epidemic station respectively.

### *Sample Collection and Microcystin Examination*

In each study site, 10 water sources (3 rivers, 3 ponds, 2 wells and 2 tapes) were randomly selected and at least eight samples were collected from each source (twice in June, July, August and September respectively). Overall, 665 water samples were collected, the samples were added 0.1 g/L sodium azide and stored at -20℃. 20% water samples ($n$=133) was randomly selected from each source and was sent to the laboratory in School of Public Health, Shanghai Medical University for microcystin examination. The samples were labeled only with numbers and the lab technicians had no knowledge of its sources. The microcystin concentrations in water sample were examined by indirect competitive ELISA (enzyme-linked immunosorbent assay) method, its detection limit was 20 pg/mL. When the microcystin concentration exceeded 50 pg/mL, the sample was considered to be microcystin positive[4].

### *Statistical Analysis*

Data were entered into computer using Visual Fox Pro 3.0 database and analyses were

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performed with SPSS 9.0. The analyses included testing for incidence rates and the assessment for relative risk (RR) by different water sources. Chi-square test was used for colorectal cancer incidence rate due to different drinking water sources. The Spearman rank correlation analysis was carried out for the association of microcystin concentration in river and pond water with colorectal cancer in each study site.

## RESULTS

### *The Incidence Rate of Colorectal Cancer and Relative Risk (RR) Assessment for Different Drinking Water Sources*

The accumulated population was 4 871 939 from 1977 to 1996 in the study site, and the average incidence rate of colorectal cancer was 8.37/100 000 in a year. The incidence rates varied in populations with different drinking water sources and increased with the order of well, tap, river and pond water sources. Compared with well water, the relative risk (RR) of different water sources for colorectal cancer was assessed and chi-square test revealed a statistically significant difference ($P$<0.01) (Table 1). However, no significant difference was observed in incidence rates between river and pond water.

TABLE 1

Incidence Rate of Colorectal Cancer and Relative Risk for Different Drinking Water Sources

| Type of Water Source | Incidence Rate (1/$10^5$) | Relative Risk | 95% CI |
|---|---|---|---|
| Well | 106/2 937 141(3.61) | - | - |
| Tap | 77/1 137 110(6.77) | 1.88[a] | 1.39-2.54 |
| River | 136/475 014(28.50) | 7.94[a] | 6.11-10.31 |
| Pond | 89/320 574(27.76) | 7.70[a] | 5.75-10.30 |

[a] $P$<0.01.

### *Microcystin Concentration in Different Water Sources*

The microcystin concentration were found to be positive only in river and pond water samples, all samples of well and tap water were negative. The MC concentration ≥ 50 pg/mL in water sample was defined as positive, the positive rate in different water sources is shown in Table 2. There was a significant difference between surface water (river and pond) and ground water (well and tap) for the average concentration of microcystin, no difference was found between river and pond or well and tap water.

TABLE 2

Positive Rate and Concentration of Microcystin in Different Water Sources

| Type of Water Source | Samples ($n$) | Positive Rate (%) | Mean Value (pg/mL) | Maximum Value (pg/mL) |
|---|---|---|---|---|
| Well | 12 | 0 | 3.73 | 9.13 |
| Tap | 17 | 0 | 4.85 | 11.34 |
| River | 69 | 36.23 | 141.08 | 1 083.43 |
| Pond | 35 | 17.14 | 106.19 | 1 937.94 |
| Total | 133 | | | |

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Since the proportion of population with different drinking water sources was similar in different towns and townships of Haining City, i.e. about 25% of the total residents in the study sites used river and pond water. The spearman correlation analysis was conducted for the relationship between the average microcystin concentration in river and pond water and the incidence rate of colorectal cancer in each study site. The correlation coefficient was 0.881 ($P$<0.01) (Table 3).

TABLE 3

Relationship between MC and Colorectal Cancer Incidence Rate [a]

| Study Site | Incidence Rate ($1/10^5$) | Concentration of MC [b] (pg/mL) |
|---|---|---|
| A | 13.14 | 321.37 |
| B | 8.15 | 158.66 |
| C | 7.82 | 203.57 |
| D | 6.44 | 219.48 |
| E | 5.85 | 49.99 |
| F | 4.38 | 70.04 |
| G | 3.57 | 34.33 |
| H | 3.57 | 21.44 |

[a] $r_s$=0.881; $p$< 0.01.
[b] The average concentration of MC in local river and pond water.

## DISCUSSION

Contamination of surface water is an increasingly severe worldwide ecological problem, which results in environmental deterioration and a public health threat. Blue-green algae is wildly spread in eutrophicated lakes, ponds and rivers of southern China, and the released algal toxins constitute a major biological source of surface water contaminant. Microcystin is a family of hepatotoxic cyclic peptides from various species of blue-green algae such as *Microcystis aeruginosa*. Epidemiological study in Qidong County revealed the incidence of hepatocellular cancer was about 8 times higher in areas where people drank pond and ditch water than that in areas where people drank well water[6]. Later Chen *et al*. and Ueno *et al*. reported contamination of higher microcystin contents in pond and ditch water might be responsible for the higher risk of liver cancer in such areas[4,7].

The association of types of drinking water and cancer incidence has been recognized by many studies abroad[8-10]. In China, Jiao *et al*. reported the relative risk (RR) of colorectal cancer in those who drank pond water for more than 25 years was 5.0 and was 10.17 in those who drank ditch water as compared with the control[2]. Chen *et al*. analyzed the relation of drinking water types with the cancer incidence of liver, lung, stomach, esophagus, colon and rectum. They found that these cancers due to drinking river and pond water had significantly higher RR value in both sexes[3]. In the current study the authors also found the higher incidence rate of colorectal cancer in those who drank river and pond water, the RR value of tap, river and pond water was 1.88, 7.94 and 7.70 respectively as compared with well water. These results were in accordance with those previously reported, the association

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between types of drinking water and colorectal cancer seemed to be quite convincing.

The authors examined the microcystin concentration in water sample of different sources, the MC positive rate in well, tap, river and pond water samples was 0%, 0%, 36.23% and 17.14% respectively. Some chlorinated tap water contained trace amount of trihalomethanes (THMs) known as carcinogen. Many ecological and case-control studies indicated that drinking chlorinated water was closely related with the incidence of colorectal, gastric and bladder cancers[11-13]. This study also revealed that the relative risk of tap water for colorectal cancer was 1.88, which was consistent with other studies.

Higher proportion of MC positive rates were detected in water samples from river and pond, this was parallel with the fact that the incidence of colorectal cancer was higher in people who drank river and pond water. Spearman rank correlation analysis showed a good correlation between the incidence rate of colorectal cancer and mean MC concentrations in river and pond water ($r_s$=0.881, $P$<0.01). These data suggested there was a close association between microcystin contamination in drinking water and incidence of colorectal cancer.

The most widely studied microcystin is microcystin-LR (MCLR), a heptapeptide containing two L-amino acids, leucine and arginine. Nishiwaki-Matsushima reported that microcystin-LR inhibited protein phosphatase type 1 and 2A (PP1 and PP2A) in rats, similar to the known cancer promotor okadaic acid[14]. Yu also showed that microcystin increased the positive foci of glutathione S-transferase placental form (GST-P) in rat liver, which was initiated with diethylnitrosamine (DEN) and/or aflatoxin[15]. Nodularin is a hepatotoxic cyclic pentapeptide isolated from one of the blue-green agal species *Nodularia spumigena*. It stimulates GST-P positive foci in rat liver more effectively than MCLR, and without initiation with DEN. Thus, it is thought that nodularin is a new carcinogen rather than a cancer promotor[16]. These results may well elucidate the possible causative role of algal toxins in hepatocarcinogenesis.

It is speculated that mycrocystin may also cause the similar changes in intestinal epithelial cells when it is ingested and penetrates the intestinal mucosa, but no direct experimental evidences support this hypothesis. Recently Zhang *et al*. revealed that microcystin-LR caused DNA damage of NK cells in rats and mice, at the same time MCLR reduced NK cell activity in mice[17]. The results suggest that microcystin may be also involved in the carcinogenesis of other organs by its general immunosuppression.

In summary, there is a close relation between microcystin contamination in drinking water and genesis of colorectal cancer, but its biological background still remains unknown. Further studies are needed to validate its etiological role and to investigate the possible mechanism.

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