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ISSN 1007-9327 CN 14-1219/R World J Gastroenterol 1998; 4(2):183-184

**Effect of various drinking water on human micronucleus frequency in high risk population of PHC**

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\*Supported by the National "8th Five-Year Plan" on the project "the Comprehensive Prevention and Treatment of Liver Cancer", No.85-914-01-04.

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**Subject headings:** liver neoplasms/etiology; carcinoma, hepatocellular/etiology; water; micronucleus tests; lymphocytes; risk factors

Liu e, Zhang QN, Li WG. Effect of various drinking water on human micronucleus frequency in high risk population of PHC. World J Gastroenterol, 1998;4(2):183-184

**INTRODUCTION**

The relationship between PHC and contamination of drinking water has been reported in some papers<sup>[1,2]</sup>. In Qidong, a PHC high-incidence area, the epidemiological investigation showed that there may be one or various carcinogens in the drinking water from various sources<sup>[3]</sup>, but these data were epidemiological results. Using a simple and effective micronucleus testing technique to evaluate environmental contamination, we observed the micronucleus effect of human peripheral lymphocytes induced by the drinking water from various sources. We reviewed and analyzed previous research data, together with the results of epidemiological investigation, in order to provide more convincing experimental evidence for the pathogenesis of PHC induced by drinking water.

**MATERIAL AND METHODS*****Experimental materials***

Water samples. Water samples were taken from house pool water (HPW), field canal water (FCW) and tap water (TW or deep well water). Water samples of these 3 types were collected in the same area, filtered, boiled and concentrated 100 times for use. Double distilled water (DDW) was used as the control. Blood samples. Obtained from 38 year old male volunteers with no diseases in physical check up.

### Experimental methods

**Preparation of micronucleus.** Put 0.3ml venous blood from volunteers into each culture flask containing heparin 50mg/L , and then add PHA 0.2ml, RPMI-1640 culture solution (contains 20% bovine serum) 4.5ml, and water samples 0.05ml-0.15ml. Keep the flask at 37 °C in an incubator for 72 hours, and shake them every 12 hours. Micronucleus was prepared according to the method reported by us [ 4 ] .

**Indices observed.** Cell growth of each experimental group each day, and micronucleus. Using a double blind test, we checked each sample under oil immersion objective to choose transformed lymphocytes which are intact and clear. Micronucleus number was counted with ‰ in 2000 lymphocytes of each blood sample. The standard to evaluate micronucleus has been reported [ 5 ] .

### Experimental grouping

Each of the 3 experimental groups (HPW, FCW and TW) was divided into 3 subgroups according to different doses of water samples (0.05ml, 0.10ml and 0.15ml) together with DDW, there were totally 10 experimental groups.

## RESULTS

### Morphologic observation of cultural cells in each experimental group

The cytotoxicity of 3 subgroups of HPW was very strong, with only a few transformed lymphocytes, and damaged membrane structure of many cells, so that the micronuclei could not be observed and the micronucleus frequency (MNF) could not be counted. The cell growth was in relatively good condition in FCW. The majority of lymphocytes were transformed with a full appearance and had intact membrane, but the cytotoxicity was enhanced with increasing doses of experimental water, and numbers of micronuclei induced were also increased. The situation of cell growth in TW groups was better than that of FCW groups. There was a weak cytotoxicity, MNF in each subgroup was lower than that of FCW groups. The cell growth condition of DDW groups was the best. There were lots of clear, transformed lymphocytes with intact membrane, abundant cytoplasm and few micronuclei.

### Dose-response relationship of MNF induced by water in each experimental group

Compared with DDW group, the MNF induced by FCW groups was the highest, and that of TW water groups was relatively lower, which rose with increasing doses of water in each experimental group (Figure 1).

**Figure 1** (PDF) Dose-response relationship of MNF induced by various water samples.

## DISCUSSION

Primary hepatocellular carcinoma (PHC) is one of ten common malignant tumors in the world. Qidong is a high-risk area of PHC in China, and its annual mortality remains at above 50/100000 . Genetic epidemiological and experimental research data showed that PHC was caused by a complex of etiologic factors, including HBV infection, liver disease history, heredity, sex, etc [ 6,7 ] . Contamination of drinking water may be one of the causes of PHC. The results of this study showed that the degree of cytotoxicity of drinking water from various sources was in the order of HPW > FCW > TW > DDW and the effect of various drinking water samples on inducing micronuclei of human peripheral lymphocytes was FCW > TW > DDW, and MNF was elevated with the increasing dose of each experimental group. Previous research data showed that the incidence rates of PHC varied in Qidong residents who drank water from various sources. The incidence was HPW (141.40/100000 ), FCW (72.32/100000 ), river water (43.45/100000), shallow well water (22.26/100000) and deep well water (11.70/100000) [ 3 ] ,

with significant difference ( $P < 0.01$ ). This suggests that MNF induced by drinking water from different sources correlates with the incidence of PHC in the population who drink the corresponding water. In other words, if the residents drink badly polluted water, the effect on micronucleus will be strong, and incidence rate of PHC will be high. Our experimental results are consistent with the results of epidemiological investigation. These data have confirmed the close relationship between drinking water and PHC incidence in the high-risk population of PHC, and the polluted drinking water may be one of the causal factors in PHC.

Recent reports showed that microcystin contents (MC), a kind of PHC carcinogen from a certain poisonous algae, was detected in the drinking water of Haimen area, a neighbour of Qidong City. The positive rates ( $> 50\text{ng/L}$ ) of MC in drinking water from various sources were 17.3% (house pool water), 31.9% (river water), 4.3% (shallow well water) and 0.0% (deep well water). The average contents of each of these water samples were 101ng/L, 160ng/L, 68ng/L and 0ng/L, respectively.

The difference was significant ( $P < 0.01$ ) [8]. The more serious the contamination of drinking water, the higher is its MC content. The relationship between MC and PHC incidence rate in residents drinking water from various sources should be further studied. It can be suggested that drinking deep well water, or improving the quality of drinking water may be of great significance in the prevention of PHC.

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