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Association between Semen Microcystin Levels and Reproductive Quality: A Cross-Sectional Study in Jiangsu and Anhui Provinces, China

Dihui Xu,[1,2] Wen Yu,[3]* Yuhan Ma,[1,2] Yang Luo,[1,2] Guanghui Xu,[1,2] Zou Xiang,[4] Yabing Chen,[1,2] and Xiaodong Han[1,2]*

[1]Immunology and Reproduction Biology Laboratory & State Key Laboratory of Analytical Chemistry for Life Science, Medical School, Nanjing University, Nanjing, China
[2]Jiangsu Key Laboratory of Molecular Medicine, Nanjing University, Nanjing, China
[3]Department of Andrology, Affiliated Drum Tower Hospital, School of Medicine, Nanjing University, Nanjing, China
[4]Department of Health Technology and Informatics, Faculty of Health and Social Sciences, Hong Kong Polytechnic University, Hong Kong, China

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Introduction

Currently, male factor infertility is a primary or contributing cause for ∼50% of couples with infertility (Agarwal et al. 2021). In recent years, microcystins (MCs), a group of cyanobacterial toxins, have gained widespread attention given the global worsening of environmental water pollution (Svirčev et al. 2019). MCs exert multiorgan toxicity in both wildlife and humans (Li et al. 2021). Indeed, toxicological studies have found that exposure to MCs can induce male infertility as reviewed by Chen et al. (Chen et al. 2016). Although previous analysis supports the notion that the testis is the second most important target organ of MCs (Chen and Xie 2005), to our knowledge, there have been no epidemiological studies regarding the effects of MCs on male reproductive health. Therefore, we conducted a cross-sectional study to assess the association between MC exposure and male reproductive quality.

Methods

Male partners of couples were enrolled at their visit to the Reproductive Medical Center, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China. The study population was heterogeneous, including infertile men and healthy men whose partners suffered from female factor infertility. The criteria for subject selection were minimal: *a*) being 20–40 y old at the time of recruitment; *b*) living for over 3 y in the Jiangsu or Anhui area, where frequent water blooms have been observed; and *c*) no potential occupational exposure to MCs. From June 2020 to January 2021, 2,588 eligible men were invited to join our study and complete the questionnaire under the guidance of a trained nurse. Of the 2,588 men, 873 were excluded as follows: 384 reported that the abstinence time was <2 or >7 d; 439 had chromosomal abnormality or experienced inflammation or surgery of the reproductive or urological system, sexually transmitted diseases, azoospermia, or a varicocele; 50 semen samples were missing. Thus, the final sample size was 1,715 in our study. We defined "cigarette smoker" as those individuals who smoked more than one cigarette per day for more than 6 consecutive months. Participants who had quit smoking for less than 6 months prior were still classified as "current smoker." We defined "alcohol drinker" as those who consumed alcohol at least once (or 50 g) per week for more than 6 consecutive months. Participants who had quit drinking for less than 6 months prior were still defined as "current alcohol drinker." Each participant provided a semen sample by masturbation. In addition, a total of 1,251 study participants provided venous blood samples for the measurement of inhibin B (924 samples) and other reproductive hormones [649 samples, including follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (T), and estradiol (E_2)], respectively. The work was approved by the Ethical Committee of Drum Tower Hospital affiliated with Nanjing University Medical School. Written informed consent was provided by each participant in this study.

MCs contained in the semen samples were examined using an enzyme-linked immunosorbent assay (ELISA) kit (Beacon Analytical Systems, Inc.) as described in a previous study (Zheng et al. 2017). A recovery test with a spiked microcystin standard in semen samples was carried out to verify the validity of the ELISA results. The average recovery was 90.2%, and the relative standard deviation was 13.1%. The semen volume was estimated based on the graduation marks on a semen collector. The sperm concentration, motility and motion parameters were assessed using a BEION S3 computer-aided sperm analysis system (CASA; Beion Medical Technology). Sperm morphology was analyzed using Shorr staining according to the World Health Organization criteria. The DNA fragmentation index (DFI) and high DNA stainability (HDS) were determined using an Accuri C5 Flow Cytometer (Becton Dickinson). Serum inhibin B was measured using an ELISA kit (Kangrun Biotech). Serum FSH, LH, PRL, T, and E_2 levels were measured by a chemiluminescence assay using an Atellica Solution system (Siemens).

A random forest model was applied to impute concentrations of MCs below the limit of detection (LOD) (0.1 μg/L) (Amit and Geman 1997). Available data for the age, body mass index (BMI), and BMI^2 of the participants were used as the parameters to impute the concentrations of MCs lower than the LOD. The missing data for baseline characteristics were imputed using the mean or mode. Multivariable linear regression was used to evaluate the changes in semen quality parameters and reproductive hormones across semen MC quartiles. Age, BMI, BMI^2, abstinence time, educational level, and smoking and alcohol consumption history were regarded as potential covariates for regression models based on the results of Spearman's rank correlation (range of the absolute value of correlations: 0.001–0.343). A 2-sided $p < 0.05$ was considered statistically significant. Because of the exploratory nature of this analysis, $p < 0.1$ was considered statistically suggestive. We used Python (version 3.7; Python Software Foundation) for the random forest model and SPSS (version 20.0; SPSS Inc.) for all other statistical analyses.

Results and Discussion

The participants were of a mean age of 30.8 y old and had a mean BMI of 24.73 kg/m^3 (Table 1). The distributions of the

*These authors contributed equally to this work.
Address correspondence to Xiaodong Han, Immunology and Reproductive Biology Laboratory, Medical School, Nanjing University, Nanjing 210093 China. Telephone: 86-025-83686497. Email: hanxd@nju.edu.cn or Yabing Chen, Immunology and Reproductive Biology Laboratory, Medical School, Nanjing University, Nanjing 210093 China. Email: ybchen916218@126.com

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semen quality parameters, reproductive hormone levels and semen MC levels are also listed in Table 1. The semen quality in our study population was similar to that in other populations (Toft et al. 2012). Semen MC quartiles were negatively associated with the total sperm count (p for trend = 0.033) (Table 2). A previous study demonstrated that MC-LR could reduce epididymal sperm counts in mice (Chen et al. 2016). We found a suggestive association between MC levels and semen volume (p for trend = 0.054). Sperm concentration was not significantly associated with MC exposure. Therefore, the decrease in the total sperm count may have been due to the MC-induced reduction of the semen volume, but not of the sperm concentration in men. For total motility, progressive motility, curvilinear velocity (VCL) and the frequency of the sperm with a normal morphology, negative trends were observed across MC quartiles (p for trend = 0.010, 0.010, 0.026, and 0.008, respectively). Moreover, MC quartiles were associated with increases in the frequency of head abnormalities (p for trend = 0.016). These results support the hypothesis that MCs might be toxins that could compromise the vitality and morphology of the sperm. Our findings are consistent with those of previous animal studies that showed that MC-LR reduced sperm motility while increased the percentage of abnormal sperm in rodents (Chen et al. 2016).

MC quartiles were found to be positively associated with the FSH levels (p for trend = 0.001) but negatively associated with the inhibin B levels (p for trend = 0.005) in this study. A previous study has also reported that increased FSH and decreased inhibin B levels were observed in infertile men (Meachem et al. 2001). Therefore, MCs might induce male infertility by disrupting the functionality of the two hormones. In addition, the association between semen MC levels and LH, PRL, T, or E_2 levels in serum was not found in this study. However, increased LH and decreased T levels were previously observed in male mice following exposure to MC-LR (Chen et al. 2016). Moreover, MC-LR induced an increase in E_2 levels in male zebrafish in a dose-dependent manner (Lin et al. 2018). The discrepancy between

Table 1. Demographics, reproductive quality parameters, and semen MC levels of the study participants in Jiangsu and Anhui provinces, China, from June 2020 to January 2021.

Variables[a]	n	Mean ± SD	Median (5th, 95th)	n (%)
Age (y)	1,715	30.8 ± 3.8	31.0 (25.0, 38.0)	—
BMI (kg/m^2)	1,708	24.73 ± 3.75	24.49 (19.03, 31.14)	—
Abstinence time (d)	1,715	—	—	—
2–4	—	—	—	1,058 (61.7)
5–7	—	—	—	657 (38.3)
Educational level	1,714	—	—	—
Junior and below	—	—	—	382 (22.3)
Senior and above	—	—	—	1,332 (77.7)
Income (yuan per person per year)	1,685	—	—	—
20,000 and below	—	—	—	224 (13.3)
20,000–50,000	—	—	—	491 (29.1)
50,000 and above	—	—	—	970 (57.6)
Smoking history	1,714	—	—	—
Current	—	—	—	802 (46.8)
Never	—	—	—	738 (43.1)
Ever	—	—	—	174 (10.2)
Alcohol consumption history	1,711	—	—	—
Current	—	—	—	504 (29.5)
Never	—	—	—	1,010 (59.0)
Ever	—	—	—	197 (11.5)
Tea/coffee consumption	1,715	—	—	—
Yes	—	—	—	1,300 (75.8)
No	—	—	—	415 (24.2)
Semen parameter	—	—	—	—
Semen volume (mL)	1,715	3.5 ± 1.4	3.2 (1.8, 6.0)	—
Sperm concentration (10^6/mL)	1,715	67.5 ± 50.7	56.6 (9.4, 160.2)	—
Total sperm count (10^6)	1,715	225.2 ± 180.4	183.7 (28.1, 548.6)	—
Total motility (%)	1,715	48.0 ± 20.0	49.1 (14.0, 79.4)	—
Progressive motility (%)	1,715	37.4 ± 18.1	37.3 (7.9, 67.2)	—
Curvilinear velocity (μm/s)	1,715	42.5 ± 9.6	42.7 (27.9, 56.5)	—
Straight-line velocity (μm/s)	1,715	19.1 ± 4.9	19.2 (11.3, 26.6)	—
Morphologically normal (%)	1,715	5.7 ± 3.1	5.5 (0.9, 10.9)	—
Abnormal head (%)	1,715	93.5 ± 3.4	93.2 (88.2, 99.1)	—
Abnormal midpiece (%)	1,715	10.3 ± 1.8	10.0 (7.7, 13.2)	—
Abnormal tail (%)	1,715	9.7 ± 2.1	9.5 (6.8, 13.6)	—
DNA fragmentation index (%)	1,134	17.11 ± 10.05	14.99 (5.92, 36.83)	—
High DNA stainability (%)	1,134	4.79 ± 4.22	3.25 (1.33, 14.22)	—
Reproductive hormone	—	—	—	—
FSH (mIU/mL)	649	4.68 ± 2.41	4.20 (1.92, 9.24)	—
LH (mIU/mL)	649	3.42 ± 1.71	3.14 (1.38, 6.46)	—
PRL (μg/L)	649	8.78 ± 4.33	7.87 (4.08, 16.31)	—
T (nmol/L)	649	11.46 ± 5.22	10.41 (4.83, 21.34)	—
E_2 (pmol/L)	649	137.06 ± 37.93	133.81 (85.50, 212.20)	—
Inhibin B (pg/mL)	924	156 ± 59.30	150.48 (78.47, 260.74)	—
MCs (μg/L)[b]	1,715	0.16 ± 0.07	0.16 (<LOD, 0.28)	—

Note: —, no data available; BMI, body mass index; E_2, estradiol; FSH, follicular stimulating hormone; LH, luteinizing hormone; LOD, limit of detection; MC, microcystin; PRL, prolactin; SD, standard deviation; T, total testosterone.

[a]A total of 7 participants had missing information on BMI, 1 on educational level, 30 on income, 1 on smoking history, and 4 on alcohol consumption history.

[b]The concentrations of MCs below the LOD were imputed with the random forest model.

Table 2. Adjusted linear regression coefficients [β (95% CI)] for changes in semen quality parameters ($n = 1{,}715$), sperm DNA damage measure ($n = 1{,}134$), reproductive hormone levels ($n = 649$), and inhibin B levels ($n = 924$) across semen MC quartiles of the study participants in Jiangsu and Anhui provinces, China, from June 2020 to January 2021.

Measure[a]	n	β coefficient (95% CI)	Measure[a]	n	β coefficient (95% CI)
Semen volume (mL)	1,715	—	Abnormal tail (%)	1,715	—
Quartile 1	428	0	Quartile 1	428	0
Quartile 2	430	−0.012 (−0.061, 0.036)	Quartile 2	430	−0.166 (−0.451, 0.118)
Quartile 3	428	−0.049 (−0.097, 0.000)	Quartile 3	428	−0.136 (−0.421, 0.149)
Quartile 4	429	−0.039 (−0.088, 0.010)	Quartile 4	429	−0.010 (−0.295, 0.276)
p for trend	0.054		p for trend	0.954	
Sperm concentration (10^6/mL)	1,715	—	DFI (%)	1,134	—
Quartile 1	428	0	Quartile 1	283	0
Quartile 2	430	−0.150 (−0.543, 0.243)	Quartile 2	284	−0.097 (−0.193, −0.002)
Quartile 3	428	−0.236 (−0.630, 0.158)	Quartile 3	284	−0.063 (−0.158, 0.032)
Quartile 4	429	−0.275 (−0.669, 0.119)	Quartile 4	283	−0.038 (−0.133, 0.058)
p for trend	0.148		p for trend	0.561	
Total sperm count (10^6)	1,715	—	HDS (%)	1,134	—
Quartile 1	428	0	Quartile 1	283	0
Quartile 2	430	−0.261 (−0.994, 0.472)	Quartile 2	284	−0.085 (−0.206, 0.036)
Quartile 3	428	−0.711 (−1.446, 0.024)	Quartile 3	284	−0.059 (−0.180, 0.061)
Quartile 4	429	−0.696 (−1.431, 0.039)	Quartile 4	283	0.018 (−0.103, 0.139)
p for trend	0.033		p for trend	0.715	
Total motility (%)	1,715	—	FSH (mIU/mL)	649	—
Quartile 1	428	0	Quartile 1	162	0
Quartile 2	430	−0.998 (−3.667, 1.672)	Quartile 2	163	0.193 (0.086, 0.300)
Quartile 3	428	−1.989 (−4.664, 0.687)	Quartile 3	162	0.161 (0.054, 0.268)
Quartile 4	429	−3.368 (−6.045, −0.690)	Quartile 4	162	0.169 (0.061, 0.277)
p for trend	0.010		p for trend	0.001	
Progressive motility (%)	1,715	—	LH (mIU/mL)	649	—
Quartile 1	428	0	Quartile 1	162	0
Quartile 2	430	−1.343 (−3.764, 1.078)	Quartile 2	163	0.065 (−0.037, 0.168)
Quartile 3	428	−2.469 (−4.896, −0.042)	Quartile 3	162	0.018 (−0.085, 0.120)
Quartile 4	429	−2.986 (−5.414, −0.558)	Quartile 4	162	0.051 (−0.052, 0.154)
p for trend	0.010		p for trend	0.415	
VCL (μm/s)	1,715	—	PRL (μg/L)	649	—
Quartile 1	428	0	Quartile 1	162	0
Quartile 2	430	−1.376 (−2.654, −0.098)	Quartile 2	163	0.011 (−0.083, 0.105)
Quartile 3	428	−2.122 (−3.402, −0.841)	Quartile 3	162	0.025 (−0.069, 0.119)
Quartile 4	429	−1.250 (−2.531, 0.032)	Quartile 4	162	0.071 (−0.024, 0.165)
p for trend	0.026		p for trend	0.165	
VSL (μm/s)	1,715	—	T (nmol/L)	649	—
Quartile 1	428	0	Quartile 1	162	0
Quartile 2	430	−0.286 (−0.941, 0.370)	Quartile 2	163	0.133 (0.041, 0.224)
Quartile 3	428	−1.047 (−1.704, −0.390)	Quartile 3	162	0.067 (−0.025, 0.158)
Quartile 4	429	−0.141 (−0.798, 0.516)	Quartile 4	162	0.046 (−0.045, 0.138)
p for trend	0.268		p for trend	0.295	
Morphologically normal (%)	1,715	—	E_2 (pmol/L)	649	—
Quartile 1	428	0	Quartile 1	162	0
Quartile 2	430	−0.061 (−0.476, 0.354)	Quartile 2	163	−0.022 (−0.081, 0.038)
Quartile 3	428	−0.271 (−0.687, 0.144)	Quartile 3	162	−0.010 (−0.070, 0.050)
Quartile 4	429	−0.531 (−0.947, −0.115)	Quartile 4	162	−0.013 (−0.073, 0.047)
p for trend	0.008		p for trend	0.685	
Abnormal head (%)	1,715	—	Inhibin B (pg/mL)	924	—
Quartile 1	428	0	Quartile 1	231	0
Quartile 2	430	0.032 (−0.421, 0.484)	Quartile 2	231	−0.374 (−0.781, 0.032)
Quartile 3	428	0.258 (−0.196, 0.711)	Quartile 3	231	−0.399 (−0.806, 0.007)
Quartile 4	429	0.521 (0.067, 0.974)	Quartile 4	231	−0.590 (−0.997, −0.183)
p for trend	0.016		p for trend	0.005	
Abnormal midpiece (%)	1,715	—	—		—
Quartile 1	428	0	—		—
Quartile 2	430	−0.045 (−0.289, 0.198)	—		—
Quartile 3	428	0.100 (−0.143, 0.344)	—		—
Quartile 4	429	0.191 (−0.053, 0.435)	—		—
p for trend	0.078		—		—

Note: For the range of MC quartiles (micrograms per liter), quartile 1, <0.12; quartile 2, 0.12–0.16; quartile 3, 0.16–0.20; and quartile 4, >0.20. Quartile 1 was used as a reference group. The concentrations of MCs below the LOD were imputed with the random forest model. The missing data for BMI were imputed with mean. The missing data for smoking history, alcohol consumption history and educational levels were imputed with mode. Estimates calculated using multiple linear regression models were adjusted for age, BMI and BMI^2 (continuous), abstinence time (2–4 d vs. 5–7 d), smoking history (current, former, and never), alcohol consumption history (current, former, and never), and educational level (junior high school and below vs. senior high school and above). The test for trend was performed by entering the median value of each quartile of semen MC levels as a continuous variable in the models. —, no data available; CI, confidence interval; DFI, DNA fragmentation index; HDS, high DNA stainability; VCL, curvilinear velocity; VSL, straight-line velocity.
[a]Semen volume, sperm concentration, total sperm count, and inhibin B were square root-transformed. DFI, HDS, FSH, LH, PRL, T, and E_2 were ln-transformed. Other parameters were not transformed.

our findings in an epidemiological study and previous animal models can possibly be explained by species specificity, lower exposure levels by humans, or other factors. Of note, MCs can impair the synthesis of reproductive hormones via disrupting the hypothalamic–pituitary–gonadal (HPG) axis (Lin et al. 2018), in addition to damaging the testis. Therefore, the relationship between reproductive hormones and MC levels in other body fluids, such as cerebrospinal fluid, is worth further investigation.

We measured MC levels in semen, which might present a more direct metric to assess the impacts of MCs on male reproductive health than that in urine or serum. Furthermore, we carried out comprehensive measurements using a relatively large sample size with strict exclusion criteria. However, there were also several limitations. First, we did not establish the relation between serum MCs and semen MCs, because toxins are transported by blood. Second, we did not consider the impact of potential exposure to other environmental pollutants, such as chemicals, secondhand smoke, and traffic exhaust. Third, further studies are required to determine whether our findings are generalizable to other populations, because all the study subjects were selected from those who visited just one reproductive medical center.

In conclusion, this is the first epidemiological investigation, to our knowledge, to evaluate the toxicity of MCs on reproductive quality in men. Our data suggested that semen MC levels were significantly associated with various male reproductive quality parameters. Given the widespread cyanobacterial pollution globally, our study can provide a reference for raising the awareness of the necessity of water bloom control.

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