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Influences of fluoride exposure in drinking water on serum androgen binding protein and testosterone of adult males

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Abstract Aim: To explore the relation between male testosterone (T) and androgen binding protein (ABP) with fluoride exposure in drinking water. **Method:** Cross-sectional study was conducted in 7 villages of a county in Henan Province as investigation points including 2 high-fluoride villages, 2 villages with water improvements and 3 control villages for collecting drinking water in all investigation points. Males who were 18 to 50 years old and born in the [abovementioned] villages were selected from investigation points as investigation subjects through group sampling. Morning urine and fasting venous blood were collected respectively. Fluoride content in drinking water and urine was determined by fluoride ion selective electrode method; serum ABP level was determined by ELISA method and serum T was determined by chemiluminescence immunoassay. **Result:** Fluoride concentration of drinking water for high-fluoride group is (2.44 ± 1.88) mg/L which is higher than (0.37 ± 0.15) mg/L of control group and higher than (0.36 ± 0.30) mg/L of improved-water group (F = 12.289, P < 0.001). Urinary fluoride concentration of high-fluoride group is (2.49 ± 1.40) mg/L which is higher than (1.04 ± 0.49) mg/L of control group and higher than (1.38 ± 0.67) mg/L of improved-water group (F = 71.563, P < 0.001); [the urinary fluoride concentration] of improved-water group is also higher than that of control group (P < 0.05). Serum ABP content of high-fluoride group is (16.01 ± 10.83) nmol/L which is lower than (27.94 ± 31.90) nmol/L of control group and lower than (22.42 ± 28.12) nmol/L of improved-water group (F = 28.807, P < 0.001). Serum T content of control group, improved-water group and high-fluoride group is (4.31 ± 1.30), (4.42 ± 1.37) and (4.74 ± 2.17) nmol/L [respectively]. The difference was of no statistical significance (F = 0.268, P = 0.765). In control group and improved-water group, negative correlation exists between serum T content and age (β = -0.238, -0.262, P < 0.05 for both groups). **Conclusion:** Environmental fluoride exposure may influence serum ABP level in males.

Key Words: fluoride; androgen binding protein; testosterone; male

High fluoride will influence sperm count and quality and damage testis, epididymis and prostate structure so as to influence male reproductive ability [1-4]. Studies [5-6] indicate that fluoride reflects certain reproductive damage and endocrine-disrupting effects through influencing multiple hormone levels of the hypothalamic-pituitary-gonadal axis in animal serum. Androgen binding protein (ABP) as an important testis protein for Sertoli cell secretion has the function of finely adjusting spermatogenesis [7]. It can promote spermatogenesis and sperm maturation. Due to its functional importance, ABP is commonly used as one marker of Sertoli cell function [8]. Animal experiments [9] have proven that fluoride will damage rat Sertoli cells; however, currently there are few reports on whether ABP secreted by Sertoli cells is influenced. The purpose of this study is to explore the influence of fluoride exposure on ABP in adult males and provide a basis for studying the effect of body ABP in sex hormone regulation through determining serum ABP and testosterone (T) levels in groups with different fluoride exposure levels.

1 Materials and methods

1.1 Selection of investigation points Seven villages were selected as investigation areas in a county of

Kaifeng City, Henan Province, including 2 endemic fluorosis villages, 2 villages with water improvement projects for decreasing fluoride levels, and 3 control villages; the economic level, natural conditions, crop types, living habits and population composition in all investigation areas were basically the same. Furthermore, there was no industrial fluoride pollution.

1.2 Selection of investigation subjects Males who were 18 to 50 years old and born in the [abovementioned] villages were selected from investigation points as investigation subjects through group sampling. People with occupational exposure to fluoride, those who worked outside for a long term, suffered from consumptive diseases and took calcium preparations for a long term or recently were excluded. Subjects composed of 175 persons in the control group, 75 persons in the improved-water group and 65 persons in the high-fluoride group were included.

1.3 Specimen collection Collecting water samples from each village: collected 16 tap water samples (control group) from control areas; collected 13 deep well water samples (high-fluoride group) from high-fluoride areas, and collected 8 tap water samples (improved-water group) from water improvement areas; after obtaining consent from investigation subjects and having the informed consent form signed, collected morning urine and fasting venous blood as marker determination. Urine was stored at -20°C for testing. When serum was separated from blood on the same day, the blood was stored under low temperature of -80°C.

1.4 Marker determination Water fluoride determination procedures were implemented strictly according to standard method (ion selective electrode method, WS/T106-1999). Determination was implemented according to standard addition method. Parallel determination was used for each sample twice; the relative error was less than 2%. Coefficient of variation was 0.4%–2.9%. Urinary fluoride determination procedures were implemented strictly according to standard method (ion selective electrode method, WS/T89-1996). The standard curve method was adopted for determination. The ELISA method was adopted for serum ABP determination; the reagent kit was provided by American R & D Company; the automatic enzyme-labeling measuring instrument was provided by American Bio-Rad Company. Chemiluminescence immunoassay was adopted for serum T determination; chemiluminescence apparatus and determination reagent kit were all provided by Zhengzhou Autobio Company. Parallel samples were set for determination and averages were taken. In addition, sampling for re-inspection was conducted at the proportion of 10%–15%.

1.5 Statistical treatment SPSS 12.0 was used to analyze the data. Single-factor variance analysis was used to analyze comparisons among water fluoride, urinary fluoride and serum ABP and T levels in the three groups. Pairwise comparisons used the LSD-t test; multiple linear regression was used to test the relation of serum T and ABP with age and urinary fluoride level for analysis. Inspection level $\alpha = 0.05$.

2 Results

2.1 Fluoride level comparison in drinking water of three groups See Table 1 for the result. Table 1 shows that the comparative differences of water fluoride levels in 3 investigation points are of statistical significance. Furthermore, the fluoride level of drinking water in the high-fluoride group was higher than that of the improved-water group and control group.

Table 1 Fluoride Level Comparison of Drinking Water for 3 Groups mg/L

Group	n	Drinking water fluoride
Control group	16	0.37 ± 0.15*
Improved-water group	8	0.36 ± 0.30*
High-fluoride group	13	2.44 ± 1.88

$F = 12.289$, $P < 0.001$; *: Comparison with high-fluoride group, $P < 0.05$.

2.2 Comparison among urinary fluoride, serum ABP and T levels of three groups See Table 2 for the result. Table 2 shows that the comparative differences of urinary fluoride levels for the three

groups are of statistical significance. Furthermore, the pairwise comparative differences are of statistical significance. The comparative differences of serum ABP levels for the three groups are of statistical significance. Furthermore, [the ABP level] of the high-fluoride group was lower than that of the control group and improved-water group. The serum T level comparison between the three groups was of no statistical significance.

Table 2 Urinary Fluoride, Serum ABP and T Level Comparison for 3 Groups

Group	n	ρ (Urinary fluoride)/(mg·L^{-1})	c (ABP)/(nmol·L^{-1})	c (T)/(nmol·L^{-1})
Control group	175	1.04 ± 0.49	27.94 ± 31.90*	4.31 ± 1.30
Improved-water group	75	1.38 ± 0.67	22.42 ± 28.12*	4.42 ± 1.37
High-fluoride group	65	2.49 ± 1.40	16.01 ± 10.83	4.74 ± 2.17
F		71.563	28.807	0.268
P		<0.001	<0.001	0.765
* For pairwise comparison between the 3 groups, $P < 0.05$ for all groups; #: Comparison with high-fluoride group, $P < 0.05$ for both groups.				

2.3 Relation of serum T and ABP with age and urinary fluoride level See Table 3 for the result. Table 3 indicates that a negative correlation exists between T and age for the control group and improved-water group.

Table 3 Relation of Serum T and ABP to Age and Urinary Fluoride Level

Group	Dependent variable	Independent variable	*β*	*t*	*P*
Control group	T	ABP	0.103	1.371	0.172
		Urinary fluoride	0.015	0.201	0.841
		Age	-0.238	3.178	0.002
	ABP	Urinary fluoride	-0.087	1.148	0.253
		Age	0.093	1.217	0.225
Improved-water group	T	ABP	-0.072	0.632	0.529
		Urinary fluoride	0.019	0.169	0.867
		Age	-0.262	2.279	0.026
	ABP	Urinary fluoride	0.040	0.339	0.375
		Age	0.066	0.561	0.576
High-fluoride group	T	ABP	0.050	0.383	0.703
		Urinary fluoride	0.201	1.578	0.120
		Age	-0.024	0.187	0.852
	ABP	Urinary fluoride	-0.084	0.673	0.503
		Age	0.221	1.775	0.081

3 Discussion

3.1 Influence of fluoride exposure on male serum ABP level ABP can buffer androgen fluctuation in tubes, leading to constant androgen release and reduced fluctuation so as to favor spermatogenesis; secondly, when ABP combines with T, it will reach the epididymis with testis fluid so as to promote sperm maturation. The lack of ABP will influence sperm count and quality. Furthermore, normal synthesis of ABP is closely related to maintaining in vivo endocrine balance and reproductive function [10-12]. Due to its functional importance, ABP is commonly used as one marker of Sertoli cell function. [This] study's results indicate that the ABP level in the high-fluoride group is lower than that of the control group and improved-water group, suggesting that long-term fluoride exposure in drinking water may cause reduced ABP level. As a kind of secretory protein, ABP will enter the endoplasmic reticulum lumen for folding and modification of polypeptide chain after synthesis, then it is transported outside of the cell for playing its role. Increased fluoride content will damage the endoplasmic reticulum so as to cause oxidative stress [13-14] and influence the folding and modification process of ABP; this may be an important reason for reduced ABP level. Many studies [15-16] indicate that heavy metals will influence ABP expression. The mentioned study result suggests that high fluoride will influence ABP level. Although the serum ABP level of the improved-water group is lower than that of the control group, the difference is of no statistical significance, prompting that such influence is reversible. When the fluoride exposure environment is removed, the damage can be repaired. Furthermore, such repair needs some time.

3.2 Influence of fluoride exposure on male serum T level T secreted by interstitial cells has broad physiological functions such as: promoting and maintaining the maturation of spermatogenesis, stimulating the development of accessory sexual organs, and promoting the appearance of male secondary sexual characteristics and maintaining their normal state. Moreover, another study has found [17] that serum T can be regarded as one of the indexes for judging the state of serious trauma, craniocerebral injury and prognosis. When T level is elevated, it worsens the reconstructive heart pathological reaction. T level is mainly regulated by luteinizing hormone (LH) and follicle-stimulating hormone which are secreted by the hypothalamus and pituitary. A study found [18] that high fluoride can decrease serum T level and increase LH level, and it presents a dose-response relation with the fluoride content. CHEN Peizhong *et al.* [19] found that, when feeding rats with sodium fluoride for 28 d, the plasma T level [began to] decrease. The result of this study shows that the comparative differences in terms of serum T levels between the three groups were of no statistical significance. The reason may be that the abovementioned studies were mainly conducted on animals, or the water fluoride level is far higher in the areas investigated [in the abovementioned studies] than that in the areas studied here. A higher water fluoride level may lead to organic disease in the testis, leading to a phenomenon whereby T level decreases with the increase of urinary fluoride level [7]. The water fluoride level in the areas studied here may not yet have greatly influenced the serum T level of the investigated population. Secondly, the body serum T level is regulated by the hypothalamic-pituitary-gonadal axis, and the multiple positive or negative feedback pathways may have comprehensive effects on the synthesis and secretion of T. In the control group and improved-water group, there is a negative correlation between the serum T level and age, which conforms to normal physiological conditions of the human body [20-21]. However, in the high-fluoride group, this correlation is not found, indicating a certain effect of fluoride exposure on male serum T [level].

In conclusion, environmental fluoride exposure may affect the ABP level in males. The serum ABP level in the high-fluoride group is evidently lower than that in the control group and improved-

water group, suggesting that fluoride may cause reproductive and endocrine disruption through affecting the ABP level.

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