Effect of Fluoride on Human Hypothalamus-Hypophysis-Testis Axis Hormones

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Objective: To determine if fluoride has an endocrine-disturbing effect on the human hypothalamus-hypophysis-testis axis hormones.

Methods: Sunying County, Kaifeng City, which has 3.89 mg/l fluoride in the drinking water, was selected as the high-fluoride area, while Shenlilou county was selected as a low-fluoride area (0.86 mg/l fluoride) for control purposes. 150 individuals who had lived in these areas for more than five years were randomly selected. Medical examinations were conducted, blood and urine samples were collected, and the serum level of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), testosterone (T) and estradiol (E2) were measured by RIA method. The level of fluoride in urine was also measured. The concentration of fluoride in the water, food, soil and air were detected by the standard methods.

Results: The concentrations of fluoride in the water, food and soil of the fluoride-polluted district were significantly higher than those of the control district ($P < 0.05$), but fluoride in the air was not detected in either district. The urinary fluoride level was significantly higher in the high-fluoride area [4.14 mg/l vs. 1.61 mg/l]. There was no significant difference in the serum level of GnRH between the fluoride-polluted district and control district ($P > 0.05$). The serum LH level in men from the fluoride-polluted district was significantly higher than the control group ($P <0.05$), while the serum level of T in men of from the fluoride-polluted district was significantly less than the control group ($P < 0.05$). Among women, there was no significant difference in the serum LH level between fluoride-polluted and control districts ($P > 0.05$), but the serum T level in women from the fluoride-polluted district was significantly higher ($P < 0.05$). There was no significant difference in the serum E2 level between the fluoride-polluted district and the control district ($P > 0.05$).

Conclusion: Fluoride could affect hormone levels of each layer of the hypothalamus-hypophysis-testis axis, and cause reproductive-endocrine disturbing effects. The reproductive-endocrine disturbing effects in males might be more severe than those in females.

Keywords: fluoride, hypothalamus-hypophysis-testis axis, gonadotropin-releasing hormone, luteinizing hormone, testosterone, estradiol

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1. Objects used for testing and methodology

1.1 Research Site

We selected Sunying Village in Kaifeng, Henan province as the primary site for research, as inhabitants of this village usually consume water containing fluoride at the level of 3.89 mg/L. This is considered a region with endemic fluorosis. Additionally, we have chosen another village whose natural surrounding environment, financial situation, demographics, agriculture, lifestyle and cuisine/food source are all similar to that of the primary research site. The control region we chose is Shenlilou Village, where inhabitants consume water containing fluoride measuring less than 1.0 mg/L. Our research found no industrial origins of pollution in either village, and inhabitants at both locations receive fluoride on a daily basis solely from drinking water.

1.2 Subjects tested and standard of inspection

By issuing a questionnaire and performing physical exams on all residents living in the target areas for 5 years or more, 150 subjects between the ages of 20 to 45 were selected. From the region with endemic fluorosis, 29 males and 38 females were selected. 37 males and 46 female subjects were selected from the control region. There are no significant differences between the age and gender distribution between the two groups ($X2 = 0.125$, $P = 0.874$). In accordance with the results of the physical exams and questionnaire, we concluded that factors such as long-term illnesses, calcium-phosphorus metabolite disorders, and other factors such as use of calcium pills would not affect the results. We have collected urine

samples (from morning period) and blood samples (taken from subjects on an empty stomach) to test the level of fluoride in the urine and hormones in the serum.

1.3 Inspection of level of exposure to fluoride

In accordance with the policy on investigating regional poisoning, we collected samples of vegetables, food items, soil, water and air in the region. We used the standard GB/T 15434-1995 filter and the fluoride ion selective electrode method to examine the level of fluoride in the air. We then used the standard of GB/T 5009.18-1996 and the disperse-fluoride ion electrode method to inspect the level of fluorine in soil, food items (dry weight) and vegetable (dry weight). We then used the standard of GB 5750-85 to inspect the level of fluorine in water and urine samples.

1.4 Daily intake of fluoride per person per day amongst inhabitant groups

As we have yet to find out the actual amount of fluoride contained in the air samples collected from the 2 target areas, we did not consider this source in calculating the total amount of fluoride intake per person per day at the target regions (5). The total amount of fluoride taken in per person per day (mg/ (person/day)) = Σ (intake of a particular food item x fluoride contained in said food item) + water consumed x concentration of water fluoride contained therein.

1.5 Inspection of serum hormones in hypothalamus and pituitary glands

We used the RIA (Radioimmunoassay) to inspect these elements. The GnRH (gonadotropin-releasing hormone) testing kit (20060430) has been supplied by the Neurobiological research unit of the Second Military Medical University of Shanghai. Testing kits for luteinizing hormone (LH), testosterone (T), Estradiol (E2) (number: 060425) have all been supplied by the China Diagnostics Medical Corporation. For conducting the RIA tests, we used the RIA calculators, GC-400y, as developed by USTC Zonkia.

1.6 Statistical analysis

We used SPSS 12.0 software and the independent sample t test to analyze data collected. Statistical significance was set at 0.05.

2. Results

2.1 Fluoride levels in the surrounding environment of the two target regions

As per the data shown in table 1 below, we found a significantly higher level of fluoride in the water, soil, food and vegetable items found in the endemic fluorosis region as compared to the control (normal) region ($P<0.05$)

2.2 Fluoride levels in urine and overall fluoride intake among inhabitants in the two regions

As shown in table 2 below, the amount of fluoride consumed by inhabitants of the region impacted by endemic fluorosis, per person on a daily basis, is significantly higher than that of the control region ($P <0.05$). The level of fluoride in the urine of those impacted by endemic fluorosis is also significantly higher than that of the control group. ($P <0.05$).

Table 1: Comparison of fluoride exposure levels in fluoride polluted district and control district

Groups	Fluoride in water (mg/L)	Fluoride in air (mg/m^3)	Fluoride in soil mg/kg		Fluoride in food items (mg/kg dry weight)	Fluoride in vegetables (mg/kg dry weight)
			Deep Level	Shallow Level		
Endemic fluorosis region	3.89 + 0.18 (1)	N/A	0.108 +/- 0.030	0.062 +/- 0.020	0.049 +/- 0.009	0.176 +/- 0.021 (1)
Control region	0.86 +/- 0.21	N/A	0.141 +/- 0.040	0.065 +/- 0.030	0.018 +/- 0.003	0.136 +/- 0.019

Note: (1) comparing against the control region $P <0.05$

Table 2: Comparison of total fluoride intake and urinary fluoride in fluoride-polluted district and control district		
Groups	**Fluoride intake (mg/d)**	**Urinary fluoride (mg/L)**
Endemic fluorosis region	12.66 +/- 0.31 (1)	4.14 +/- 1.12 (1)
Normal control region	2.38 +/- 0.19	1.61 +/- 0.68
Note: (1) comparing against the control region P <0.05		

Table 3: Comparison of serum hormone levels in fluoride-polluted and control districts (x +/- s)						
Gender	**Group**	**N**	**GnRH (ng/L)**	**LH (μg/L)**	**T (μg/L)**	**E2 (ng/L)**
Male	Endemic region	29	4.65 +/- 0.60	18.36 +/- 2.26 (1)	186.12 +/- 15.89 (1)	99.13 +/- 15.43
	Control region	37	4.54 +/- 0.78	9.86 +/- 3.36	205.11 +- 18.31	86.05 +/- 11.05
Female	Endemic region	38	4.83 +/- 0.81	10.47 +/- 2.26	70.49 +/- 5.17 (1)	48.55 +/- 3.77
	Control region	46	4.75 +/- 0.73	8.51 +/- 1.55	32.82 +/- 8.61	51.15 +/- 8.56
Note: (1) comparing against the control region P <0.058						

2.3 Serum hormone levels in subjects from the two regions

From Table 3 below it can be clearly seen that regardless of gender, there is no significant difference between the serum level of GnRH in the subjects from the endemic region and control region. However, the serum LH level in male subjects from the epidemic region is significantly higher than that of the control region (P<0.05), whereas the serum T is significantly lower (P<0.05). In female subjects, the serum LH level does not have a significant difference from that of the control region, but the T level is significantly higher (P<0.05). The serum E2 level in both the male and female subjects from the endemic region is not significantly different than that of the control region.

3. Discussion

3.1 Significant differences in fluoride exposure levels between the two regions

The present research team has concluded that the level of fluoride in water in Sunying Village averages 3.89 mg/L, which is considered a relatively severe epidemic region, and its inhabitants are exposed to fluoride from drinking water, soil, and common agricultural goods to a significantly higher degree than that of the control region (P<0.05). The overall intake of fluoride in inhabitants from the endemic region is significantly higher than that of the control group (P<0.05), and is higher than the national standard of 3.0-3.5 mg (per person per day). The consumption of fluoride via the consumption of drinking water in both regions, however, is significantly higher than the consumption of fluoride via food items. This indicates that the region is an endemic fluorosis region because of large amounts of fluoride in the drinking water. The level of fluoride contained in the urine samples taken from the epidemic region is significant increased (P<0.05). The levels are representative of those who have been continually over-consuming fluoride, and the effects of these levels can be readily seen in various bodily functions, organs etc.

3.2 The impact of fluoride exposure on the subjects' blood serum GnRM

GnRH is secreted from the hypothalamus and has an important regulating effect on the reproductive functions of the human body. It can increase the secretion of serum hormones in the pituitary glands and can suppress gonadal hormones. As per research conducted by Hongwei Zhong et al., an excess of GnRH dimer can suppress the formation of T in male swine and can impede the development of sexual organs (6). Other animal testing has proved that using GnRH 1h anti-agents produces a clear increase in blood serum T in male lab rats, but the E2 blood serum level in female lab rats does not show any significant changes. The results of the current study show that the serum GnRH level in subjects from the region impacted by endemic fluorosis is elevated, but the difference is not statistically significant. The reason for this could be that the subjects' have a complex hormonal adjustment system, which allows the subject to maintain GnRH at regular levels. When the level of fluoride in water measures at 3.89 mg//L, we are not yet to able to see an impact on the serum level of GnRH.

3.3 The impact of high fluoride exposure on the formation and release of LH pituitary hormone

LH is a glycoprotein capable of regulating the serum hormones secreted by the serum pituitary glands. External elements and substances can impact the formation and release of LH in the body. The present research has found that the level of LH in the body of male subjects from the epidemic fluorosis region is much higher than that of the control region ($P<0.05$). The reason could be that fluoride is directly responsible for the formation and release of LH. It could also be due to a long-term effect of fluoride in reducing the number of GnRH receptors which results in the disintegration of GnRH and phosphoinositide. Other literature has shown that when the pathways where the cell G q/11 passes through are frequently under activation, the cell might selectively shut down or slow down the pathway where the cell PLC-PKC-Ca2+ passes through, which in turn lowers the level of sensitivity of serum cells in reaction to the function of GnRH (7). As LH no longer reacts to the regulation of GnRH, and as serum T significantly lowers in level, this will result in a higher level of negative regulation of LH in the subject's body.

The present research also shows that while the level of LH in the female subjects from the endemic region is higher than in the control group, the differences between the two groups are not significant. This is likely due to the impact of the female menstrual cycle. Female subjects at different periods of the menstrual cycle might be experiencing a largely different level of LH in their blood serum. The menstrual cycle therefore likely obscures or cancels out the differences of serum LH between the female subjects of the two groups.

3.4 The impact of high fluoride exposure on T and E2

The present research has shown that the level of LH in male subjects in the endemic region is much higher than that of the control region ($P<0.05$), whereas the level of T is much lower than the control region ($P<0.05$). This result is consistent with many previous research projects which have found that a large amount of fluoride can interfere with the normal function of the pituitary-testicular axis. High levels of fluoride can also be damaging to the functionality and make-up of leydig cells, which can impact testosterone formation and secretion. Fluoride, via oxidative stress, can result in a negative impact and/or damage in the development of the testicles and/or creation of sperm (8-9), and can suppress glycolysis. This causes a reduction in the by-product produced from the tricarboxylic acid cycle, which then further suppresses the formation of steroid hormones. The present research also indicates that the reduction of the T/LH ratio in male subjects in the endemic area is quite significant. This is indicative of the interference caused by fluoride, which causes the lowering of the T/LH ratio, which results in turn in an impairment of the sperm-formation function.

The present research also shows that the level of blood serum E2 in both male and female subjects of the high fluoride region is not significantly different than that of the control group. The reason for this could be that the main path to forming estradiol in the human body comes from post-metabolite T being aromatized. Sertoli cells can also secrete a small amount of E2. In regions where fluoride in water measures at 3.89 mg/L, the dismembering of fluoride into aromatizing enzymes

could further suppress steroid hormones and turn them into estradiol.

3.5 The effect of high fluoride exposure on the internal secretion of serum hormones in the human body

In summary, based on the results gathered from this investigation, we can conclude that the level of LH in the male subjects from the endemic region are significantly elevated, whereas that of the female subjects have had no significant change. The level of T in the male subjects has decreased while that of the female subjects has increased. There are no significant changes in the serum E2 level in either male or female subjects from the endemic region. It is important to remind the reader that fluoride can interfere with the level of reproductive hormones found in the human body, which results in clear interference with the internal secretion of reproductive organs. This interference particularly impacts males as opposed to female subjects.

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