

Initial Study of Cognitive Function Impairment as Caused by Chronic Fluorosis

SHAO, Quanli¹, WANG, Yanan², LI, Liangwen³, LI, Jian⁴

¹CGuiyang Medical College, Guiyang 550004, China

²Biochemical Department of Stockholm University, Rasundouagen 0046-16967

³Nayong County Hospital of Guizhou, Nayong 553300, Guizhou Province, China

⁴Clinical Office of Guizhou Military Area, Guiyang 550002, Guizhou Province, China

Abstract:

Objective: To investigate whether an impairment in cognitive function exists in patients diagnosed with fluoride poisoning as well as its biological basis.

Methods: Individuals suffering from fluoride poisoning from a high fluorine region were selected for the study group, while healthy individuals from a non-endemic region were selected as the control group. Using specific psychological methods, the indices of blood oxidative stress were also tested with the following: Thiobarbituric acid reaction (TBA) for serum lipid peroxide (LPO), Ellman's for reduced glutathione (GSH) in serum, nitrite method for superoxide dismutase (SOD) in red blood cells, and the Dithiobis-nitrobenzoic acid (DTNB) method for glutathione peroxidase (GSH-Px) in serum. As to determining the amount of NO in the subjects, this was done through testing the nitrous and nitrate (NO₂-/NO₃-) in serum.

Results: Between the study group and the control group, significant differences were observed in the data gathered from the psychological tests. Big differences were found in the tests of language fluency, recognition, similarity, associative learning, and working memory (digit span test). The total failure numbers from the psychological tests and the concentration of NO demonstrated a significant positive correlation, while the similarity test showed a significant negative correlation. SOD activity showed a significant negative correlation with the similarity and digit span tests.

Conclusion: The results suggest that some cognitive function limitations exist in those suffering from chronic fluoride poisoning, and its biologic basis may be related to the levels of SOD and NO.

Keywords: Chronic Fluoride Poisoning; Cognitive Function; Oxidative Stress

Endemic fluorosis can cause overall pathological injury to the human body [1], and yet its pathogenesis continues to remain unclear. Only a relatively few number of studies discussing the influence of endemic fluorosis on the patients' level of intelligence are presently available. This study examined the influence of chronic fluoride poisoning on the intelligence of patients in the endemic area of Guizhou Province. We focused in particular on the correlation between the patients' cognitive functions and oxidative stress as a result of continuous excessive exposure to fluoride (which could cause potential oxidative increase in the brain tissues as seen in tests performed on lab rats).

1. Subjects and Methods

1.1 Subjects

1.1.1 *Study Group.* Individuals suffering from fluoride poisoning were selected from an endemic area

in Bijie City, Guizhou Province, which is not an area impacted by iodine-deficiency.

Selection criteria. Individuals with fluoride poisoning were identified as those with a diagnosis of dental fluorosis, a decrease in bone density upon X-ray examination, a thickening of the periosteum, calcification of the skeletal muscle and ligaments, and a decrease in physical fitness and cholinesterase activity, etc. Selection was further limited to subjects between the ages of 30 and 50, who had been suffering from the disease for over 2 years, who had not yet accepted systemic treatment or used antioxidants, who had been excluded from experiencing symptoms of mental disorders caused by stunted mental development and/or organic and somatic diseases of the brain. 49 individuals (37 males, 12 females) were selected in total, all of whom were farmers. The average age was 42±6 years and the average length of education was 10±2 years. 20 people from the group obtained a high school degree, 25

completed junior high, and 4 completed their primary studies. The average duration of the disease among these subjects was 32 ±6 months.

1.1.2 Control group. The subjects were all healthy residents from the Tongren area of Guizhou Province (an area not impacted by neither iodine-deficiency nor endemic fluorosis). Selection criteria. Control subjects could not have any bodily and/or mental diseases and their 1st degree relatives could not have any history of neural and/or mental diseases. We selected controls whose gender, age and education level were not statistically different than that of the study group (P<0.05). 39 people were selected and all were farmers; 26 of this group were males and 13 were females. The average age was 43±6, and the average years of completed schooling was 10±3 years. 19 of the controls obtained a high school degree, 18 finished junior high, and 2 completed primary school.

1.2 Methods

Neural psychological tests: The study group took the tests one week after being diagnosed with fluoride poisoning, while the control group completed the tests immediately after being selected. The tests that were administered were as follows. The revised version of the Wechsler Adult Intelligence Scale (WAIS-RC), a test used to determine the IQ score of each subject; associative learning (AL) test: AL is a type of language memory and is relevant to the left anterior frontal and temporal lobes; Digital Span (DS) test: DS may be used as a method to test a subject’s working memory; Similarity test: it is commonly believed that the similarity test is relevant to

the left temporal lobe and the left prefrontal lobe; Speech fluency test (SFT): SFT is a receptive tool used to estimate the injury inflicted on the prefrontal lobe, especially in the Brocas area; Comprehension test: a test used to check the subject’s ability to summarize things.

1.3 Determination of the Blood Oxidative Stress Index

Preparation of specimen: All subjects had 5ml of venous blood samples taken at about 9 am while on an empty stomach; of this 5ml sample, the 50µl of whole blood was cleaned with 5ml of saline solution and centrifuged at 3000 r/min centrifuge. The supernatant was removed, with only the red blood cells remaining. 1 ml of whole blood was extracted and then placed into a heparin anticoagulation tube; the remainder of the whole blood extract was then placed into the centrifuge tube, subjected to a 10 minute shower with the temperature measuring at 37°C and centrifuged 3000 r/min centrifuge. The supernatant (serum) was then extracted. All specimens were kept in a refrigerator of -70°C and placed aside for a one-off test.

Test process: We used the thiobarbituric acid reaction (TBA) test for serum lipid peroxide (LPO), the Ellman’s method to examine the amount of reduced glutathione (GSH) in the serum, the nitrite method for erythrocyte superoxide dismutase (SOD) activity, and the dithiobis-nitrobenzoic acid (DTNB) method to test the whole blood glutathione peroxidase (GSH-Px) activity. As for NO (Nitric oxide), since it is an active substance whose condition makes it impossible to directly determine its level of concentration in the serum samples, it was determined by checking the level of nitrous and nitrate

Table 1: Results of the Neural Psychological Tests of Study Group and Control Group

Group	Cases	WAIS-RC				Cognitive Function Test				
		Language Scores	Operation Scores	Combined Scores	Total IQ Value	Speech Fluency	Recognition	Similarity	Digit Span	Associative Learning
Study Group	41	69-81	48-54 **	104-132	78-110 (average)	36.2±10.2 **	9.1±4.2 **	10.5±2.8△	11.8±2.1 *	19.0±6.9
Control Group	37	73-83	52-59	126-142	109-118 (average high)	54.3±15.0	13.1±2.4	12.3±1.9	13.8±3.1	22.9±5.5

Note: compared with control group, * P<0.05, ** P<0.01

(NO₂⁻/NO₃⁻) in serum. Copper cadmium reduction method was used to figure out the amount of NO₂⁻/NO₃⁻.

2. Results

2.1 Neural Psychological Testing

See Table 1.

2.2 Oxidative Stress Index

Among the parameters of oxidative stress between the study group and the control group, the SOD and NO results were found to have statistically significant differences (P<0.01), whereas the remaining indices did not have statistically significant differences. Please refer to Table 2 for more details.

2.2 A simplistic correlative analysis of the study group's cognitive function and oxidative stress index

The concentration of NO found demonstrates a significant but negative correlation to the similarity test. The samples' SOD activity showed a significant but negative correlation with the similarity and digit span tests. This indicates that the injury caused to the cognitive function found in patients diagnosed with fluoride poisoning is relevant to SOD and NO. For more details, please refer to Table 3.

3. Discussion

Among those suffering from chronic fluorosis, research has found that certain injuries occur to bodily functions, with the biological basis for these injuries relevant to the increase in the oxidative stress level of brain tissue caused by long-term consumption of excess fluorine.[2] In particular, active oxygen produced by oxidative stress

Table 2: Comparison of oxidative stress indices of study group and control group (x±s)

Group	Number of Cases	SOD (Hb)	GSH (n mol/g Hb)	GSH-Px (U/g)	LPO (m mol/L)	NO
Study group	41	679±81*	1.32 ±0.20	0.076±0.014	2.69±0.60	53±31*
Control group	39	789±79	1.40±0.40	0.079±0.020	2.71±0.60	46±18

Note: compared with control group, *P<0.01

Table 3: Correlation of Oxidative Stress Indices to Cognitive Function Test Results of Study Group

Group	Cognitive Function Test				
	Speech fluency	Recognition	Similarity	Digit span	Associative learning
SOD	-0.177	-0.137	-0.403*	-0.420*	-0.269
GSH	-0.189	-0.162	-0.086	-0.097	-0.121
GSH-Px	-0.091	-0.087	-0.004	-0.117	-0.152
LPO	-0.022	-0.107	-0.094	-0.231	-0.363
NO	-0.079	-0.091	-0.431*	-0.306*	-0.161

Note: the figure in the table is correlation (r value); the hypothesis test for r value, * P<0.05

and the resulting injury to secondary cells play a critical role in the development of nerve cells. Under regular conditions, the body has a complete antioxidant defense system. SOD is an important antioxidant enzyme in the body. This study demonstrates that SOD activity has a negative correlation with the body's executive functions and working memory. This trend towards increased levels of oxidation in the bodies of those suffering from chronic fluorosis indicates that the body's defense system against free radical agents is impaired. This, in turn, could cause the peroxidation of cell lipids and DNA damage, which could injure certain channels inside the neural cells, leading to the defects of certain cognitive functions found in fluorosis patients.[2] Meanwhile, the compensatory mechanisms in the body, and the compensatory activity of the SOD (which acts as an important antioxidant enzyme) increases, implying that the biological basis of the cognitive deficits among patients with chronic fluorosis is correlated to SOD.

An excessive amount of NO has cytotoxic effects on the nervous system[3]. The effect of NO has brought researchers' attention on the process by which cell damage results from active oxygenic elements. In this study, we found that the concentration of NO in blood serum presents a negative correlation with the body's executive functions, which indicates that the cognitive deficits in patients with chronic fluorosis may be related to the level of oxidative stress. With the increased concentration of NO, damage is inflicted on the cell. Certain neural channels are particularly impacted by such damage, which can result in a decline of the cognitive functions executed by these affected neural channels.

In this study, only SOD and NO were found to be related to the cognitive function results among the chronic fluorosis patients, thereby indicating that these two oxidative stress parameters likely had a hand in impairing the patients' cognitive function. In the process of cell damage induced by oxidative stress, SOD and NO are the two parameters found to be most closely related to the damage[4]. Studies show that SOD has a clear role in the reduction of NO's poisoning effects on nerves. In the cerebral cortex and neostriatum, NO-positive neurons have high levels of SOD. This defense mechanism helps to regulate against impairments in the human antioxidant system. This is the reason why cognitive function among those suffering from chronic fluorosis decreases

at a relatively static or slow speed, which makes it hard to detect.

References

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