Using Urinary Metallic Elements as the Indicator for the Assessment of Intervention on Children with Autism Spectrum Disorder

Chen Li, Kangwei Shen, Jinli Qiu, Dongchuan Yu, and Xuejun Kang

***Abstract*—Autism spectrum disorder (ASD) is a neurodevelopmental disease. The pathology of ASD was unclear. Some existed hypotheses are from nutritional and environmental aspects. urinary metallic elements have close connections with these factors. There is not a specific therapy for ASD. Intervention is a conventional treatment to alleviate the behavioral problems of ASD patients. After intervention, the effects require evaluation. This paper reported a preliminary attempt in using urinary elements as an indicator for the assessment of the intervention effects on a small sample of ASD children. The results showed a homodromous alteration of urinary elements and the behavioral performances. It was suggested that the ratio of urinary beneficial elements to hazardous elements could serve as a potential indicator for the assessment of the intervention effects on ASD children.**

***Index Terms*—Assessment, autism spectrum disorder, intervention, metallic elements.**

I. Introduction

Autism spectrum disorder (ASD) is a typical neurodevelopmental disease. According to the latest epidemiological investigation, the prevalence of ASD in children under 8 is 1.4% in America [1], 1% and 2.6% in two major cities in pre-school children in China [2], [3]. Therefore, ASD has influenced a large amount of people around the world.

The pathology of ASD is unclear. Nutritional and environmental factors are among the possible causes. Metallic elements are closely connected with the two factors. Some of the metallic elements are crucial for human health and function of different systems, which are called beneficial elements (BE), such as calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), chromium (Cr) and selenium (Se). Some elements are toxic to human body, which are called hazardous elements (HE), such as aluminium (Al), cadmium (Cd), lead (Pb) and arsenic (As). Many metallic elements play an important role in the maintenance of human health. The imbalance of beneficial elements and hazardous elements is possible to cause a wide variety of physical and psychiatric conditions [4]. For ASD children, there are studies reporting the significantly reduced ratio of beneficial elements to hazardous elements in comparison with normal children [5].

Up to now, there are still no specific therapies for ASD patients. The widely used method is the intervention on the apparent behavioral problems, which could alleviate the symptoms of ASD patients [6]. After the intervention, it is necessary to examine the intervention effects. A conventional approach is to compare the behavioral performances before and after intervention [7].

With the development of interdisciplinary studies, some researchers found the relationship between the severity of ASD and some biochemical indicators, some of these biochemical indicators were defined as biomarkers. Therefore, biochemical analysis has also been applied in the assessment of intervention effects in some aspects in ASD patients [8-9].

Considering the association of urinary metallic elements with nutritional and environmental factors in the pathology ASD, the purpose of the present study was to compare the urinary ratios of beneficial elements to hazardous elements like before and after a period of intervention in ASD children. To our knowledge, no previous study has investigated the urinary metallic elements during the intervention of ASD children.

II. Method

A. Participants

Participants were recruited through several physical projects in Nanjing Mingxin kindergarten, which had a specific division for the education on ASD children. All the children were diagnosed with ASD by psychiatrists and pediatricians according to DSM-5 [10] and kept a record in the kindergarten. Children were excluded if they had other psychiatric disorders or another chronic medical comorbid condition. Children who had history of genetic diseases were also excluded. All the participants were confirmed of having

Manuscript received December 28, 2016; revised May 1, 2017. This work was supported in part by the Open Project Program of Key Laboratory of Child Development and Learning Science of Ministry of Education, Southeast University (No. CDLS-2016-04), Maternal and Child Health Research Project in Jiangsu Province (F201303), Heshengyuan Maternal and Child Nutrition and Health Research Project (2014FYH022), the Fundamental Research Funds for the Central Universities, Colleges and Universities in Jiangsu Province Plans to Graduate Research and Innovation Projects (SJZZ_0026, KYZZ_0070).

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doi: 10.18178/ijssh.2017.7.5.841

no medication or dietary intervention in three months.

Furthermore, all the children accepted a one-to-one educational intervention lasted for a month, the intervention includes the trainings on children's behavior and cognition using programs on tablet computers [11].

The guardians of all the participants were explained the aim and procedures of the experiment. They all signed informed consent forms. This work was in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The experiment was approved by the ethics committee of Zhongda Hospital, Southeast University.

B. Specimen

Children's first morning urine were chosen for the determination of urinary metallic elements. Their urines were collected by their guardians on a normal school day. After children awaked in the morning at about 8 am and urinated, their urines were collected using aseptic tubes and sent to researchers when their guardians took them to their kindergartens at about 9 am in the morning.

The c first morning urine specimens were collected twice before and after the intervention respectively for all children. All the urine specimens were frozen at -20℃ immediately till determination.

C. Determination of Urinary Metallic Elements

The preparation method for urine specimens and assay for urinary metallic elements were quoted from a reported ICP-MS method [12]. In short, urine samples were firstly centrifuged, then 1mL was taken out to dilute with nitric acid, internal standard solution and deionized water to 5 mL. These prepared samples were injected into ICP-MS for analysis. The limit of quantification of this method were from 0.04 to 0.5 ng/mL.

III. Results

The concentrations of urinary metallic elements in pre-intervention specimens were shown in Table I.

TABLE I: Urinary Metallic Elements Concentrations (Pre-intervention)

No.	Beneficial elements (ppm)	Hazardous elements (ppm)	ratio
1	14.23	12.56	1.13
2	21.36	67.59	0.32
3	11.20	11.38	0.98
4	7.19	12.87	0.56
5	14.60	36.33	0.40

The intervention on five ASD children lasted for a month. The intervention was focused on their inhibitory control ability, control ability, working memory and cognitive flexibility [11].

The concentrations of urinary metallic elements in post-intervention specimens were listed in Table II.

TABLE II: Urinary Metallic Elements Concentrations (Post-Intervention)

No.	Beneficial elements (ppm)	Hazardous elements (ppm)	ratio
1	18.82	8.37	2.25
2	40.86	67.90	0.60
3	34.10	15.46	2.21
4	3.44	7.79	0.44
5	19.93	17.37	1.15

In order to compare the equilibrium of the urinary BE and HE, the ratio of urinary BE to HE was computed in the urine specimens before and after intervention. The results were shown in Fig. 1.

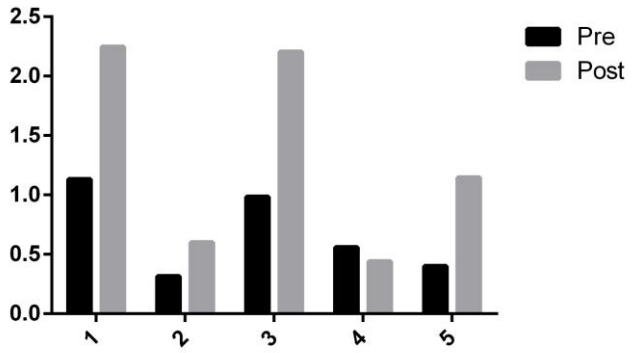

Fig. 1. Ratio of urinary BE to HE.

It was clearly demonstrated that four children showed obvious increase in the ratio of urinary BE to HE after the intervention, while, one children showed slight decrease.

IV. Discussion

In the prior studies, increased proportions of urinary trace elements (beneficial elements) were found in ASD children [5]. In human body, the beneficial elements play roles in many metabolic activities. While, the hazardous elements are toxic and harmful to human health [13]. In this experiment, we found increased ratio of urinary BE to HE in most of the ASD children after they received a period of intervention. From the BE/HE level, the alteration was tending to a healthier orientation.

The five ASD children accepted the intervention for a month. Before and after the intervention, their performances on inhibitory control ability, cognition flexibility and working memory are also evaluated. The evaluations were quantified by measuring their accuracies on inhibitory control ability and cognition flexibility, maximum number of consecutive correct answer, and reaction times in three different attention tasks, results were shown in figure 2 and figure 3 [11].

From the two figures, the accuracies on inhibitory control ability and cognition flexibility were improved, the maximum number of consecutive correct answer increased, while the completion times required in attention tasks dropped dramatically. These results meant their abilities on these aforementioned aspects were enhanced after the intervention.

Combining these results with the prevent experiment, the alteration of the ratio of urinary BE to HE was in the same direction with the change of the behavioral performances in ASD children. The results were presented in figure 4.

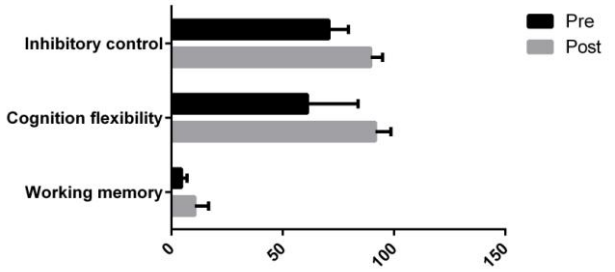

Fig. 2. Performance of executive function tasks. (Evaluation index: Accuracies for inhibitory control and cognition flexibility, maximum number of consecutive correct answer for working memory; Experiment paradigms: Go/No-Go paradigm for inhibitory control [14], one-back paradigm for working memory [15]).

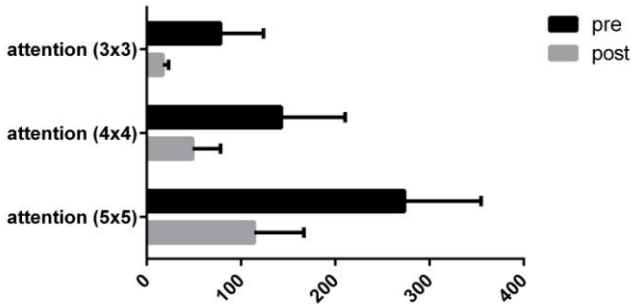

Fig. 3. Reaction times of attention tasks. (Experiment paradigm: Schulte Grid test) [16].

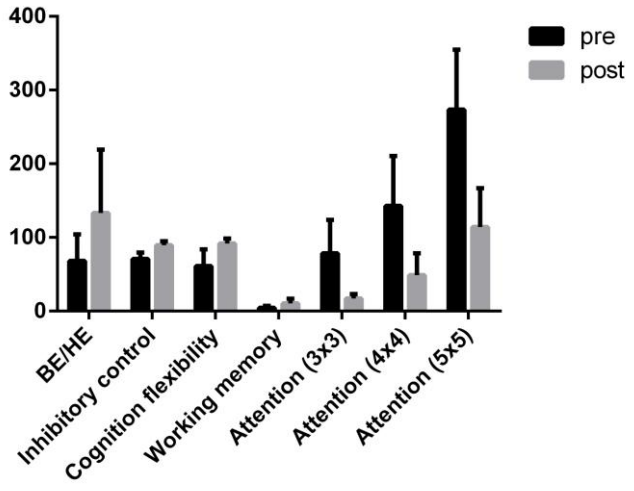

Fig. 4. Alteration of metallic elements and behavioral performances after intervention.

It was suggested that the enhancement of the BE/HE and improvement of behavioral performances synchronized after intervention. ASD is a disease, which have abnormalities in multiple systems [17]. The intervention on the behaviors, executive function and cognitive function was possible to influence some other aspects.

In this experiment, the homodromous relationship between the alteration of urinary ratio of BE to HE and the change of apparent behavioral performance indicated the potential association of the metallic elements with some behavioral functions in human.

A previous study has confirmed the relationship between the imbalance of metallic elements with the normal function of neurons and immune system [18], [19]. And the ratio of Zn/Cu has been proposed as a diagnostic indicator for ASD [20]. In this study, the homodromous improvement of BE/HE and behavioral performances had the similar relationship with the previous report. Therefore, urinary metallic elements had the potential to serve as an index for the assessment of intervention effects on ASD children.

V. Conclusion

Our study detected obviously increased ratio of urinary beneficial elements to hazardous elements after the intervention on ASD children, which showed the homodromous improvement with behavioral performances. It was suggested that the ratio of urinary BE to HE was related with behaviors, executive function and cognitive function. The investigation of urinary metallic elements in ASD patients offered a simple and objective method for the assessment for the effects of intervention conducted on them.

Acknowledgment

This study is supported by the Open Project Program of Key Laboratory of Child Development and Learning Science of Ministry of Education, Southeast University (No. CDLS-2016-04)， Maternal and Child Health Research Project in Jiangsu Province (F201303), Heshengyuan Maternal and Child Nutrition and Health Research Project (2014FYH022)，the Fundamental Research Funds for the Central Universities, Colleges and Universities in Jiangsu Province Plans to Graduate Research and Innovation Projects (SJZZ_0026, KYZZ_0070).

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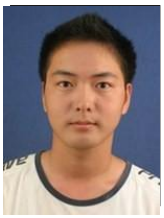

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