The Relationship between Hair Zinc and Lead Levels and Clinical Features of Attention-Deficit Hyperactivity Disorder

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Objectives : The goal of this study was to examine the association between zinc and lead level and symptoms of attention-deficit hyperactivity disorder (ADHD) among Korean children.

Methods : A total of 89 clinic-referred children participated in the study (ADHD group=45, control group=44). The participants were 5−15 years old, and were mainly from urban areas of Seoul, Korea. ADHD was diagnosed using the Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version. We excluded children with a comorbid psychiatric disorder, medical illness requiring medication, or a prior history of taking ADHD medication. In order to evaluate the severity of ADHD symptoms, parents' Korean ADHD Rating Scale (K-ARS) was used. The ADHD diagnostic system (ADS) was used for evaluation of the severity of inattention and impulsivity. All participants completed the intelligence test and hair mineral analysis. Multiple regression analysis was used to examine the effect of hair zinc and lead levels on the K-ARS and ADS. We measured the predictive ability of the zinc and lead levels using logistic regression analysis.

Results : The lead level explained the score for omission errors, commission errors, and response time SD in visual ADS in the ADHD group (adjusted R^2=.243, p<.01, adjusted R^2=.362, p<.01, and adjusted R^2=.275, p<.01), the score for omission errors of auditory ADS in ADHD group (adjusted R^2=.407, p<.01) and the entire group (adjusted R^2=.292, p<.01). Zinc was significantly explanatory for the K-ARS scores for the entire group (adjusted R^2=.248, p<.001) and the ADHD group (adjusted R^2=.247, p<.05).

Conclusion : These findings suggest a possible role of zinc and lead in ADHD. Lead concentration in hair samples affected the ADS scores, and this was more prominent in children with ADHD. Children with ADHD had a lower zinc concentration in their hair, and the zinc concentration in hair showed negative correlation with the K-ARS score.

KEY WORDS : Attention-Deficit Hyperactivity Disorder · Zinc and Lead · ADS · Hair Analysis.

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a very common disease in 3−5% of school-age children. The disease is characterized by the symptoms of inattention and hyperactivity-impulsivity due to various causes. The cause of ADHD is still unknown. In previous studies, probandwise concordance rates of ADHD have ranged from 50% to 80% for monozygotic twins and have been approximately 30% for dizygotic twins, and the study by Biederman and Faraone[1] estimated the average hereditary ratio to be 0.77. Despite the well-known high contribution of hereditary factors,

attention continues to be paid to the environmental factors of ADHD. Nigg[2] stated that the cause of this is the increased medical, systematic, and customers' interest in the environmental factors that affect pediatric diseases such as obesity, asthma, cancer, learning disabilities, and behavioral disorders in modern society. Another cause of the interest in the environmental factors of ADHD is the emphasis on the interplay between genes and the environment, in which environmental factors affect the onset of genetic diseases.[2] The environmental factors that affect ADHD such as drinking, smoking, exposure to environmental toxic substances, perinatal complications, accumulations of heavy metals, mineral deficiency, and various food additives during pregnancy have been studied.

Among the environmental factors of ADHD, the risk of increased concentration of lead in the body is well known, and has long been studied. Lead was widely used as an ingredient of gasoline additives and paint in the past, but its use has

gradually been limited as the renal toxicity of lead, its correlations to lung cancer, reproductive functions, and mental retardation in children have been reported.[3] In 1985, the Centers for Disease Control and Prevention specified that the blood lead level should be less than 25μg/dL, but they decreased it to less than 10μg/dL in 1991. However, as there are research reports that show that there is a correlation between pediatric mental retardation and impulsivity even at the plasma concentration of lead that is less than 10μg/dL, opinions that advise re-examination of the limits of plasma concentrations of lead are dominant.[4] In 2006, Braun et al.[5] conducted a study on 4,704 children from the age of 4 to 15 years in the USA, and reported that the incidence of ADHD was higher by a factor of 4.1 (95% confidence interval 1.2−14) when the blood lead concentration exceeded 2μg/dL. This study once again aroused awareness that the current limits of blood lead concentration were not appropriate.

There is increasing concern about the adverse effects of heavy metals and mineral deficiencies on pediatric cognitive functions as more children are diagnosed with ADHD and learning disabilities in Korea. Reports about the effects of environmental hormones, food additives, and heavy metals in toys and school supplies feature commonly in the mass media. A recent study analyzed the correlation between the internal lead concentration and ADHD in Korean children. In the study by Ha et al.[6] in 2008, blood lead and mercury concentrations were measured in 1,778 elementary school students from 6 cities, and their correlation with the Conners Parents Rating Scale (CPRS) was investigated. The results showed no correlation between mercury and CPRS scores, but lead showed significant correlation with CPRS scores. This study was a large-scale research on children in the community, and had the advantage of considering confounding variables such as gender, age, region, parent's education level, economic level, and parent's psychiatric disorders. However, this study relied on the parent's assessment of the symptoms and did not control for or consider intelligence or coexisting diseases.[6]

To assess the effects of lead on cognitive functions more objectively, studies have been conducted using neuropsychological tests such as the Continuous Performance Test (CPT), stopping tasks, and digit span. Walkowiak et al.[7] in 1998, Chiodo et al.[8] in 2007, and Nicolescu et al.[9] in 2010 used CPT for impulsivity and attention to analyze the effects of lead on cognitive functions. Walkowiak et al.[7] measured CPT and blood lead concentration in 384 6-year-old children, and reported on the correlation between the blood lead level and false alarm error. However, the study by Chiodo et al.[8] did

not find any correlation between blood lead concentration and impulsivity, and the study by Nicolescu et al.[9] showed a small degree of correlation.

In addition to lead-related problems in ADHD, mineral deficiencies are also getting attention as a cause of ADHD. Zinc is the most actively studied chemical, and children with ADHD have consistently been reported to have a lower zinc concentration in the blood compared to control groups. There are study reports on zinc affecting the emission of lead, and that zinc and lead concentrations in the blood are inversely proportional. This has led to suggestions that injections of high doses of zinc should be given to groups with high lead concentrations.[10]

For the measurement of heavy metals and minerals, the blood, saliva, urine, hair, bones, and nails can be used. Hair testing has increased gradually from 1980, and the US Environmental Protection Agency reported its significance as a measurement of heavy metal contamination in humans. In pediatric psychiatry, there are studies that measured the lead levels by using hair samples from children with autism and ADHD.[11,12] Hammer et al.[13] pointed out that hair sampling is appropriate for long-term lead exposure research because lead accumulates more in the bones or hair than in the blood, and it tightly couples with the protein chiol (-SH) in hair follicles. Recently, hair samples are being used for the measurement of zinc and magnesium since hair reflects the nutritional status of a relatively long period of time, and it is preferred in the studies on children, in whom blood sampling is difficult.

The purpose of this study was to find the clinical characteristics associated with the accumulation of zinc and lead deficiency in children with ADHD who visited the pediatric psychiatry department. According to previous studies, the levels of heavy metals and minerals depend on race, age, gender, and regional differences. In order to minimize these differences, this study was conducted on Koreans whose age and gender were controlled for, to measure the heavy metal and mineral concentrations in the hair, and the patient group was compared to the control group.

Methods

1. Subjects

1) Patient group
For this study, children between the ages of 5 and 15, who had been diagnosed with ADHD by pediatric psychiatrists, were recruited as subjects. The children were diagnosed with

ADHD using the Diagnostic and Statistical Manual of Mental Disorder, fourth edition (DSM-IV), Kiddie-Schedule for Affective Disorders and the Schizophrenia-Present and Lifetime Version-Korean version (K-SADS-PL-K), and the severity of the ADHD symptoms were measured by the Korean ADHD Rating Scale (K-ARS) for parents. K-SADS-PL-K was performed to evaluate any coexisting diseases, and the computerized ADHD Diagnostic System (ADS) was used to evaluate attention and inhibitory control. In order to eliminate the influence of intelligence, the Wechsler intelligence scale for children (WISC-III) was used, and children with an intelligence quotient (IQ) of 70 or less were excluded from the study. Children with psychiatric disorders in addition to major physical illnesses, convulsive disorders, psychosis, pervasive developmental disorder, and who had been medically treated for ADHD within 6 months after the research participation had begun were also excluded from the study. For an accurate hair test, subjects who had dyed hair or had gotten a perm within the last 3 weeks, and the subjects who were taking supplements that contain minerals were also excluded. The patients (children or their parents) recruited for this study signed the informed consent form before the hair mineral test and psychological tests, and they were informed that the test materials were being utilized for statistical research purposes only. A total of 45 ADHD children were recruited, but 2 of them did not meet the criteria of the study, and the other 2 did not complete the psychological testing, and therefore they were excluded from the analysis.

2) Healthy control group

The healthy control group was recruited through advertising (hospital website and posters). Subjects were children between the ages of 5 and 15, with no psychiatric disease or major physical diseases. Subjects were recruited considering their age, gender, and region. Participants were identified with no psychiatric disorders and major physical disorders by the psychiatrist's interview and K-SADS-PL-K. In order to eliminate the influence of intelligence, the WISC-III was performed by clinical psychology professionals, and children with an IQ of less than 70 were excluded. For an accurate hair test, subjects who had dyed or permed their hair within the recent 3 weeks and the ones taking supplements that contain minerals were excluded from the study. The K-ARS for parents or ADS and the hair mineral test were performed by the recruited healthy control group, and an informed consent form about the use of the intelligence test data and statistical data for research purposes was signed. A total of 44 healthy subjects were recruited, but 2 of them were excluded because they did not complete psychological testing due to the geographical distance of their location.

The institutional review board approved this study, and a total of 41 children with ADHD and 42 healthy control subjects were included for the analysis.

2. Research tool

1) K-SADS-PL-K

The Korean translated version of the K-SADS-PL, the original of which was developed by Kaufman et al.,[14] is a semistructured interview designed to evaluate the current and lifelong conditions of 32 child and adolescent psychiatric diseases that are based on the DSM-IV. Its reliability and validity have been reported for ADHD, hostile defiance disorder, tic disorders, depressive disorders, and anxiety disorders. Each question is rated with 0 point for 'no information', 1 point for 'none', 2 points for 'below threshold', and 3 points for 'threshold'.

2) Korean ADHD Rating Scale (K-ARS)

This study used the K-ARS for the evaluation of children's inattention and hyperactivity/impulsivity symptoms of ADHD. K-ARS was developed by Du Paul[15] in 1991, and was designed to evaluate the symptoms of ADHD in school-age children. There are 18 redeveloped questions that correspond to the ADHD diagnostic criteria of the DSM-IV. Each question is rated with 0 point for 'never or not true', 1 point for 'sometimes', 2 points for 'often', and 3 points for 'very often'. The questions in odd numbers measured carelessness, and the even numbered questions measured hyperactivity-impulsivity. The ARS was translated into Korean, and So et al.[16] studied the reliability and validity of the ADHD evaluation scale for parents and teachers.

3) ADHD Diagnostic System (ADS)

The use of two types of continuous performance tests (CPT), the visual and auditory CPT, increases the accuracy of diagnosis of ADHD for children. ADS are composed of visual and auditory tests because children with ADHD have difficulty in visual or auditory information processing. The basic variables measured in ADS were as follows.

- Omission error : measures inattention (carelessness), and refers to when the subject did not respond to a target stimulus
- Commission error : measures impulsivity and disinhibition, and occurs when the subject responds to a non-target stimulus
- Response time : the time to accurately respond to a target stimulus (msec). People with ADHD problems take a longer

time to respond to the target stimuli compared to normal people. This variable is very important in diagnosing ADHD.

- Standard deviation (SD) of the response time : this is the SD of the time to accurately respond, and it measures the inconsistencies of the response. People with ADHD problems can concentrate for a short time, but their response becomes inconsistent with a longer time interval.

3. Hair mineral test

This study used the hair mineral test to measure zinc and lead levels in hair. For the hair mineral test, participating children and their parents were asked to refrain from dying or perming the child's hair for 3 weeks before the study, to not wash the hair or use any type of hair products within 3 hours before visiting. To reduce anxiety and improve the children's cooperation during the hair sampling, their caregivers were asked to stay with them. Hair was sampled from the head in the length of 3cm from the hair root, and in the weight of 0.15gm by using stainless steel scissors, and a scale was used to weigh the sample. A hair mineral analysis firm called TEI (Trace Elements Inc., Addison, TX, USA) was commissioned for the analysis.

The procedure of the hair sample processing was as follows. The sample was cut in the size of 3mm or below, weighed, then inserted in a 50mL acid-washed polypropylene centrifuge. Then, ultrapure trace-metal grade HNO_3 was added. After 5 minutes of centrifugation, the samples were placed in a CEM Mars 5 Plus Microwave Digestion apparatus. The sample was allowed to stand for 20 minutes at 70°C, and then the temperature was increased up to 115°C for another 15 minutes. Then the samples were cooled at room temperature, then were diluted with a deionized water/gold solution and

mixed well. The mineral content was analyzed by using ICP-MS [inductive coupled plasma-mass spectrometry (SciexElan 6100, Perkin-Elmer, Houston, TX, USA)].

4. Data analysis

The demographic variables, zinc and lead levels in hair, the K-ARS score, the ADS score and intelligence between the ADHD group and control group were analyzed through the chi-square test, Fisher's exact test, t-test and analysis of variance (ANOVA). Also a partial correlation analysis was conducted to examine the relationship between zinc and lead levels in hair, the K-ARS score, the ADS score and intelligence.

Based on the variables extracted through the analysis above, we carried out the analysis as follows. First, we performed a logistic regression analysis to analyze which factor had a risk of causing ADHD. After that, multiple regression analysis was conducted to see how much the zinc and lead levels could explain the K-ARS score and ADS score. In the case of the multiple regression analysis, we set both the K-ARS score and ADS score as dependent variables, and lead, zinc, age, gender, intelligence and body mass index (BMI) percentage as independent variables. Gender was processed as a dummy variable.

Statistical Package for the Social Sciences (SPSS) 18.0 version (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The significance level was set at $p<.05$.

Results

1. Demographic characteristics of children with ADHD and the control group

There was no statistically significant difference in age, gen-

Table 1. Sample summary statistics for ADHD and controls : demographic variables

	Control (N=42)	ADHD (N=41)	p
Age (month), mean (SD)	119.71 (34.97)	115.68 (35.67)	NS*
Birth weight (kg), mean (SD)	3.33 (0.39)	3.30 (0.43)	NS*
BMI percentage, mean (SD)	56.05 (30.78)	48.03 (32.45)	NS*
Male, N (%)	37 (88.1)	34 (82.9)	NS†
Residential area (Seoul), N (%)	34 (81.0)	34 (82.9)	NS‡
Education level of mother (>12 years), N (%)	32 (78.0)	24 (60.0)	NS†
Education level of father (>12 years), N (%)	37 (90.2)	24 (61.5)	.003†
SES, N (%)			NS§
High	8 (19.0)	5 (12.2)	
Middle	28 (66.7)	23 (56.1)	
Low	6 (14.3)	13 (31.7)	
Housewife, N (%)	12 (28.6)	8 (19.5)	NS‡

＊: statistical significance is based on t-test, as appropriate, † : statistical significance is based on Fisher's exact test, as appropriate, ‡ : statistical significance is based on chi-square test, as appropriate, § : statistical significance is based on analysis of variance, as appropriate. ADHD : attention-deficit hyperactivity disorder, BMI : body mass index, SES : socioeconomic status, NS : not significant

Table 2. Sample summary statistics for ADHD and controls : K-ARS, ADS, IQ, hair lead and zinc level

	Control, mean (SD)	ADHD, mean (SD)	p
K-ARS	3.75 (3.69)	27.81 (12.38)	<.001
Full scale IQ	112.14 (11.40)	100.90 (15.37)	<.001
Verbal IQ	115.50 (11.33)	105.29 (14.92)	.001
Performance IQ	105.12 (13.15)	95.83 (15.46)	.004
Visual ADS			
Omission	49.73 (12.94)	72.31 (36.46)	<.001
Commission	55.38 (17.03)	65.82 (21.45)	.016
Response time	50.69 (10.59)	55.07 (10.90)	NS
SD of response time	58.64 (19.55)	78.56 (38.95)	.005
Auditory ADS			
Omission	46.64 (8.56)	59.87 (19.86)	<.001
Commission	44.59 (6.85)	51.19 (14.91)	.011
Response time	56.76 (11.89)	63.02 (14.69)	.036
SD of response time	54.78 (13.39)	68.80 (16.81)	<.001
Zinc (mg/100g of hair)	18.69 (23.59)	12.90 (7.26)	NS
Lead (mg/100g of hair)	0.21 (0.24)	0.14 (0.07)	NS

Statistical significance is based on t-test. ADHD : attention-deficit hyperactivity disorder, K-ARS : Korean ADHD Rating Scale, ADS : ADHD Diagnostic System, IQ : intelligence quotient, NS : not significant

der, birth weight and BMI between the ADHD children group and the control group (Table 1). Most of the children who participated in the study lived in Seoul (ADHD : 34, 82.9%, controls : 34, 81.0%), and 5 of the children lived in Gyeonggi-do, 1 in Chungcheongnam-do, and 1 in Jeollabuk-do. The parents' education level was found to be higher in the control group compared to the ADHD group. There were 37 fathers who had more than 12 years of education (90.2%), and 32 in the control group, but 24 fathers (61.5%) in the ADHD children group had these levels of education (p<.01). There was no significant difference in the economic levels, and housewife mothers between the two groups. Economic levels were divided into 'high, midhigh, middle, lowermid, and low'. For the analysis, 'high' and 'midhigh' were put as 'high', 'mid' as 'mid', and 'lowermid' and 'low' as 'low'.

2. Difference in clinical characteristics of children with ADHD and the control group

The ADHD children group had higher K-ARS and ADS scores compared to the control group (Table 2). For intelligence, the control group had an average of 112.14 (±11.40), and ADHD children had 100.90 (±15.37), and this was statistically significant (p<.001). Average zinc and lead levels were 12.90mg/100g of hair (±7.26), 0.14mg/100g of hair (±0.07) in the ADHD group, and 18.69mg/100g of hair (±23.59) and 0.21mg/100g of hair (±0.24) in the control group, which were both higher in the control group, but no significance was found.

Logistic regression analysis was performed to estimate

the risk factors of ADHD, and significantly different variables were used as independent variables. The results significantly showed that intelligence and zinc were the predictive variables for ADHD (odds ratio=1.112, 95% confidence interval=1.036−1.194, p=.003) (odds ratio=1.244, 95% confidence interval=1.053−1.471, p=.010) (Table 3).

3. Partial correlation analysis of K-ARS, ADS, intelligence, zinc, and lead

Partial correlation analysis was performed to investigate the correlation between the K-ARS total score, the subscales of ADS, zinc, and lead by controlling for the demographic variables, BMI percentage. For analysis of the K-ARS total score and ADS, intelligence was also controlled, and for the ADHD group analysis, coexisting diseases were also controlled.

Results of the partial correlation analysis showed that there was a positive correlation between lead and auditory false alarm (r=.336, p<.01), and a negative correlation between zinc and the K-ARS total score(r=−.459, p<.001). In the control group, there was only a significant correlation between the lead concentration and omitted auditory stimulus(r=.587, p<.01). When the ADHD group and the control group were divided for analysis, the ADHD group had a significantly positive correlation of omitted auditory stimulus, commission errors, and SD of the response time with the lead concentration, and zinc had a negative correlation with the K-ARS total score (Table 4).

Table 3. Result of logistic regression analysis

| | B | SE | Wald | df | p value | Exp (B) | 95%CI for EXP (B) | |
							Lower	Upper
Age	−.006	.013	.209	1	.648	.994	.970	1.019
BMI percentage	.015	.012	1.453	1	.228	1.015	.991	1.039
Birth weight	.288	.793	.132	1	.717	1.333	.282	6.311
Full scale IQ	.106	.036	8.708	1	.003	1.112	1.036	1.194
Zinc	.219	.085	6.590	1	.010	1.244	1.053	1.471
Lead	2.857	2.537	1.267	1	.260	17.401	.120	2514.440
Sex	.029	.923	.001	1	.975	1.030	.169	6.284
Constant	−15.713	5.501	8.160	1	.004	.000		

BMI : body mass index, IQ : intelligence quotient, B : logistic regression coefficients, SE : standard error, EXP : expected value, SD : standard deviation, RT : response time

Table 4. Correlation of K-ARS, ADS, IQ, hair lead and zinc level in ADHD group

| | Visual ADS | | | | Auditory ADS | | | | K-ARS | Intelligence quotient | | |
	Omission	Commission	RT mean	RT SD	Omission	Commission	RT mean	RT SD		Verbal	Performance	Total
Zinc	−.084	.059	−.057	−.085	.091	.171	.109	.076	−.727[†]	−.087	−.330	−.210
Lead	−.599*	.647[†]	−.161	−.661[†]	.756[†]	.553*	−.010	.640[†]	−.087	.076	−.168	−.032

* : p<.05, † : p<.01. K-ARS : Korean ADHD Rating Scale, ADS : ADHD Diagnostic System, ADHD : attention-deficit hyperactivity disorder, RT SD : reaction time standard deviation

Table 5. Results from multiple regression analysis for ADS with hair lead level as an independent variable in the ADHD group

	Dependant variable	R^2	F	p	Predictor	Beta	t	p
Visual ADS	Omission	.389	2.658	.039	Lead	.500	2.936	.007
	Commission	.485	3.932	.007	Lead	.614	3.929	.001
	RT SD	.415	2.957	.025	Lead	.466	2.799	.010
Auditory ADS	Commission	.521	4.541	.003	Lead	.410	2.720	.012
					Total IQ	-.366	-2.258	.033

ADS : ADHD Diagnostic System, ADHD : attention-deficit hyperactivity disorder, IQ : intelligence quotient, RT SD : reaction time standard deviation

Table 6. Results from multiple regression analysis for ADS with hair lead level as an independent variable in the total group

	Dependant variable	R^2	F	p	Predictor	Beta	t	p
Auditory ADS	Commission	.356	5.532	<.01	Total IQ	−.457	−3.921	<.01
					Lead	.358	3.178	.002

ADS : ADHD Diagnostic System, IQ : intelligence quotient

4. Variables affecting K-ARS, ADS

To evaluate how well zinc and lead explained the scores of K-ARS and ADS, multiple regression analysis was performed with the variables that had the ability to affect the K-ARS and ADS. In the patient group, lead was significantly explanatory for the ADS values except for the visual response time, auditory omission error scores, response time and response time SD (Table 5), and the entire group lead was significantly explanatory only for the auditory commission error scores (Table 6). Zinc was significantly explanatory for the K-ARS scores in the entire group and the ADHD group (Table 7, 8).

Discussion

This was a comparison study investigating the effects of lead and zinc in children with ADHD. The results comparing the variables with the control group were as follows. 1) The increased lead concentration in the ADHD group affected the increase in visual omission, commission errors, response time SD, and the scores in auditory false alarm, 2) the increased lead concentration affected the scores in auditory false alarm in the total group, and 3) zinc was a factor associated with an increased risk of ADHD, and had a significant negative correlation with the K-ARS.

Table 7. Results from the multiple regression analysis for K-ARS with hair zinc level as an independent variable in the ADHD group

Dependant variable	R^2	F	p	Predictor	Beta	t	p
K-ARS	.393	2.696	.037	Zn	−.623	−2.671	.013
				Age	.579	2.381	.025

K-ARS : Korean ADHD Rating Scale, ADHD : attention-deficit hyperactivity disorder, Zn : zinc

Table 8. Results from multiple regression analysis for K-ARS with hair zinc level as an independent variable in the total group

Dependant variable	R^2	F	p	Predictor	Beta	t	p
K-ARS	.316	4.628	.001	Total IQ	−.542	−4.507	<.01
				Zn	−.363	−2.740	.008

K-ARS : Korean ADHD Rating Scale, IQ : intelligence quotient, Zn : zinc

The mechanism of lead affecting cognitive functions has long been studied. Lead is known to affect synaptogenesis, postsynaptic N-methyl-D-aspartate receptor sensitivity, calcium-mediated events, neurotransmitter dopamine release, and mitochondrial activity.[17] Children's brains are in constant development, and are very sensitive to the effects of lead. In an animal study, prenatal exposure to lead was found to reduce the turnover of dopamine in the striatum of dopamine type 2 receptors, and caused the symptoms of ADHD.[18]

There have been studies on the correlation between lead and the difficulty of concentration, impulsivity, and attention, but the measurement of the ADHD symptoms mainly relied on the subjective evaluation of the parents and teachers of the children. Although the impact on intelligence has been continually reported, there have been limitations of the studies, such as the uncontrolled-for effects of intelligence.[3] This study considered these limitations, and thus professional consulting with pediatric psychiatrists, K-SADS-PL, and psychological testing were conducted. For the statistical analysis, the age, gender, parents' education, region, birth weight, BMI percentage, mother's occupational status, socioeconomic status, and intelligence that affected ADS in the multivariate analysis, coexisting diseases in children with ADHD, and the testing hours (morning/afternoon) were all controlled.

In 2008, Nigg et al.[19] recruited 150 children aged 8−17 years (mean age=14 years ; 53 control subjects, 47 ADHD-predominantly inattentive type, 50 ADHD-combined type) for assessment of the blood lead concentration, intelligence, and stop signal task performance. This study used the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Epidemiologic Version, and recruited children with ADHD who had been diagnosed by pediatric psychiatrists, as well as healthy subjects for the control group, and tried to investigate how intelligence affected the relationship between lead concentration and AD-HD symptoms through a path model.[19] In 2010, Nigg et al.[20] recruited 236 children and reported on the blood lead level and the correlation between hyperactivity-impulsivity and the lead concentration level, and stated that the average lead concentration was 0.76 and the highest level shown was 2.2. This reconfirmed that lead had effects at a low concentration.

The results of the present study showed that the concentration of lead in the hair was associated with the scores of ADS. There was a positive correlation between the concentration of lead in the hair and the auditory commission error score seen in the overall group, consistent with the results of previous studies.

Another characteristic of this study was that zinc was included in the analysis. As mentioned in the introduction, a relatively large number of studies have shown a reduction in the concentration of zinc in children with ADHD. Ward et al.[21] found significantly lower levels of zinc in the samples of urine, hair, blood, and finger nails of 20 of the age and gender-matched control subjects.[21] They also found lowered blood zinc and iron by comparing 486 hyperactive boys and 172 control subjects.[21] The study by Arnold et al.[22] revealed that the children who had zinc deficiency showed more severe ADHD symptoms according to the parents and teachers' evaluation scales, and this was related to omission errors.[22]

In the present study, the concentration of zinc in the hair was higher in the control group. Independent t-test comparing the patient group and the control group did not show a significant difference, but logistic regression analysis showed that zinc deficiency was a significant risk factor of ADHD. Also, in multiple regression analysis, zinc was significantly explanatory for the K-ARS scores in the total group and the ADHD group, and the results corresponded with those of the previous study that showed that zinc deficiency was related to ADHD symptoms.

In the previous study, zinc was considered to help with the emission of lead, and thus when there was a lack of zinc, the accumulation of lead was considered to be a cause of the symptoms of ADHD.[10] However, in recent years a hypothesis that the binding sites of the dopamine transporters are not

filled due to the lack of zinc, and this in turn increases the activation of dopamine transporter, which causes a decrease in dopamine, leading to the symptoms of inattention, is gaining attention.[23] There have been studies that investigated the use of zinc and stimulants in improving the symptoms of ADHD. Bilici et al.,[24] Akhondzadeh et al.,[25] and Arnold et al.[26] conducted randomized double-blind trials, but the results did not correspond with each other, and therefore, more research is needed in the future.

Another problem is the difficulty of accurate measurement in zinc studies in children with ADHD. Concerns about the impact of minerals in the human body are increasing, but the measurement is not easy. Most of the studies used blood analysis, but the measurement of levels in the blood does not reflect the long-term status of zinc, and is also easily susceptible to being influenced by infection, stress, and eating. Therefore, various other tests using samples of hair, urine, saliva, cellular or non-cellular fractions, and plasma protein have been conducted in addition to the blood tests. King[27] stated that measuring more than 2 samples is accurate.

In this study zinc and lead were measured by using hair. Hair testing is relatively well known as a tool for examining long-term exposure to heavy metals. A hair test can investigate the long-term exposure to heavy metals and the recent changes in the last six months of minerals. Therefore, it has been widely used in measuring long-term exposure to heavy metals or the nutritional status. In 1978, hair testing was announced as a substitute for blood and urine tests,[28] and in 1980, the United States Environmental Protection Agency reported the usefulness of hair testing in heavy metal contamination in the human body, and since then, its use has been gradually increasing. A large number of studies have measured the lead concentration in the blood. However, blood lead has a half-life of 1 to 2 months, whereas hair reflects the concentration of 3 to 5 months ; thus, hair appears to be useful in measuring longer exposure. In 2011, Grabeklis et al.[29] recruited 263 workers and measured their exposure to metals during work by using samples of blood, serum, urine, and hair. The results showed that hair testing was the most sensitive method for measuring heavy metals.[29]

Hair sampling is less invasive than blood samples, and the measurement can be easily repeated ; therefore, it can be a good substitute for sampling in children. Hair sampling is used in the evaluation of atopic diseases, pediatric diabetes, renal syndrome, and many other diseases where heavy metals and minerals are measured, and the results show good correlation. The pediatric psychiatric department has also performed hair testing to investigate the correlation between

intelligence and heavy metals, and the relationship between heavy metals in hair samples of children with ADHD and autistic children.[11,12]

This study has the other following limitations : First, this study was a cross-sectional study, and thus the relationship between zinc and lead levels and ADHD could not be explained by a cause and effect relationship. For example, the possibility of the behavioral characteristics of children with ADHD causing increased or decreased levels of zinc and lead needs to be considered. Second, in this study, iron was not included in the analysis. Iron is known to help emissions of zinc and lead from the body, and iron deficiency is associated with the severity of the symptoms of ADHD, and there are study reports that the level of iron is lower in ADHD patients compared to the controls. Similarly, supplementing iron in the children with ADHD is reported to improve their symptoms.[30]

This study used hair to measure the minerals. It was difficult to measure iron and magnesium by using blood ; Thus hair and saliva were used for the measurement, but blood samples are known to be the most accurate test for iron.[1] In future research, using two or more samples to analyze heavy metals and minerals would result in a more credible study.

Conclusion

This study investigated the relationship between ADHD and the concentrations of zinc and lead by using hair samples. Lead concentration in hair samples affected the ADS scores, and this was more prominent in the ADHD group. Children with ADHD had a lower zinc concentration in their hair, and the zinc concentration in the hair was negatively correlated with the K-ARS score.

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