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Double-blind, placebo-controlled study of zinc sulfate in the treatment of attention deficit hyperactivity disorder

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Abstract

Background: The most commonly used medications for attention deficit hyperactivity disorder (ADHD) are the psychostimulants. There is, however, considerable awareness in alternative, nonstimulant therapies, because some patients respond poorly to stimulants or are unable to tolerate them. Some studies suggest that deficiency of zinc play a substantial role in the aetiopathogenesis of ADHD. Therefore, to assess the efficacy of zinc sulfate we conducted treatment trial. *Methods*: Patients with a primary DSM-IV diagnosis of ADHD ($N=400$; 72 girls, 328 boys, mean age $=9.61 \pm 1.7$) were randomly assigned in a 1:1 ratio to 12 weeks of double-blind treatment with zinc sulfate ($n=202$) (150 mg/day) or placebo ($n=198$). Efficacy was assessed with the Attention Deficit Hyperactivity Disorder Scale (ADHDS), Conners Teacher Questionnaire, and DuPaul Parent Ratings of ADHD. Primary efficacy variables were differences from baseline to endpoint (last observation carried forward) in mean ADHDS and Conners Teacher Questionnaire scores between the zinc sulfate and the placebo groups. Safety evaluations included monitoring of adverse events, vital signs and clinical laboratory values. *Results*: Zinc sulfate was statistically superior to placebo in reducing both hyperactive, impulsive and impaired socialization symptoms, but not in reducing attention deficiency symptoms, as assessed by ADHDS. However, full therapeutic response rates of the zinc and placebo groups remained 28.7% and 20%, respectively. It was determined that the hyperactivity, impulsivity and socialization scores displayed significant decrease in patients of older age and high BMI score with low zinc and free fatty acids (FFA) levels. Zinc sulfate was well tolerated and associated with a low rate of side effect. *Conclusions*: Zinc monotherapy was significantly superior to placebo in reducing symptoms of hyperactivity, impulsivity and impaired socialization in patients with ADHD. Although by themselves, these findings may not be sufficient, it may well be considered that zinc treatment appears to be an efficacious treatment for ADHD patients having older age and high BMI score with low zinc and FFA levels.

Keywords: ADHD; Fatty acids; Treatment; Zinc

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a serious, high-incidence disorder that impairs academic learning, disrupts social and peer relations, and can greatly disturb functioning within the home and at school. ADHD is characterized by symptoms of inappropriate inattention, impulsivity, and overactivity, according to DSM criteria (American Psychiatric Association, 1994). Although many studies have been carried out regarding the aetiology of ADHD, no definite cause of the illness has yet been determined.

In the recent years, some research into the role of trace elements in enlightening the aetiology of ADHD had been

Abbreviations: ADHD, Attention deficit hyperactivity disorder; ADHDS, Attention Deficit Hyperactivity Disorder Scale; ADHDS-A, Attention Deficit Hyperactivity Disorder Scale Attention Deficit Subscale; ADHDS-H, Attention Deficit Hyperactivity Disorder Scale Hyperactivity Subscale; ADHDS-I, Attention Deficit Hyperactivity Disorder Scale Impulsivity Subscale; ADHDS-S, Attention Deficit Hyperactivity Disorder Scale Impaired Socialization Subscale; ACTQ-A, Ankara Conners Teacher Questionnaire Attention Deficit Subscale; ACTQ-H, Ankara Conners Teacher Questionnaire Hyperactivity Subscale; ACTQ-C, Ankara Conners Teacher Questionnaire Conduct Subscale; BMI, body mass index; DuPaul, DuPaul Parent Ratings of ADHD; EFA, Essential fatty acid; FFA, free fatty acids; TACTQ, Turkish Adaptation of Conners Teacher.

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conducted. Most notable among the studies are the ones particularly examining the relationship between zinc and ADHD. Both animal and human study suggest that involvement of zinc deficiency plays a major role in hyperactivity (Golub et al., 1996). Human zinc deficiency syndrome leads to concentration impairment and jitters (Agget and Harries, 1979), and can delay cognitive development (Black, 1998). Some investigators have reported that zinc was found to be significantly deficient in ADHD compared to controls (Bekaroglu et al., 1996; Kozielec et al., 1994); and we concluded that zinc deficiency might play a role in the aetiopathogenesis of ADHD (Bekaroglu et al., 1996). In addition we reported previously a significant correlation between serum zinc and serum free fatty acid (FFA) levels in patients with ADHD, who had significantly lower FFA and zinc than those of healthy controls (Bekaroglu et al., 1996). With those findings, it may suggest that zinc and fatty acids may be used in ADHD treatment.

In spite of the good response of many patients with ADHD to stimulant drugs, a substantial percent do not respond to or develop significant side effects from stimulants. For this reason, new investigations have been initiated for ADHD treatment. As an example, it has been shown in some research that if ephamol is given to children with ADHD having a lack of essential fatty acids, a rise is observed in the plasma levels of dihomo-δ-linolenic acid, while some patients recover from hyperactivity (Mitchell et al., 1987). Similarly, zinc treatment has been considered to show positive results on various symptoms in ADHD patients with zinc deficiency. Zinc supplementation is hypothetically supported by systematic case-control data, but no systematic clinical trial. If it is not to consider the coadministration of zinc with other medication in the treatment of ADHD (Arnold et al., 2000), as far as we know, there is no double-blind placebo-controlled study on the effect of the administration of zinc in the treatment of ADHD.

Using that study, the authors undertook a confirmatory, randomised, placebo-controlled, parallel-group study to more definitively address the issue of efficacy of zinc for ADHD symptoms. Our primary hypothesis was that zinc administration would lead to significantly greater reduction in ADHD symptoms than would placebo, as determined by using the Attention Deficit Hyperactivity Disorder Scale (ADHDS). Secondary aims were addressed to possible patterns of response, safety and tolerability.

2. Methods and materials

2.1. Study design

This study was conducted in the Department of Psychiatry, Karadeniz Technical University (KTU), Trabzon, Turkey. During the school year from 1996–1997, a two-phase surveillance was conducted on a total of 21979 primary school children throughout the primary schools from Trabzon province (10 805 girls and 11 174 boys) between the ages of 6 and 14 (the mean 9.05 ± 1.9 years) in the first five grades.

2.1.1. Phase I

All primary school class teachers were given an "ADHD Determination Form for Teachers" (Bilici et al., unpublished data) comprising 46 questions, to be filled out for every child in the class separately. In this form adapted by us, diagnosis criteria proposed in DSM-IV (American Psychiatric Association, 1994) were simplified and converted to "yes or no" question format. Apart from this, the 14-items DuPaul Parents Ratings of ADHD (DuPaul, 1991) was reorganized, given the name of "ADHD Evaluation Form for Parents" and distributed to his or her mother or father such that they would only be required to answer in "yes or no" format. Both forms were then recollected from parents and teachers after a period of 1 week. In the results obtained, those providing positive answers to at least 30 questions in the 46-question "ADHD Determination Form for Teachers" and positive answers to at least 10 questions in the 14-question "ADHD Evaluation Form for Parents" were taken into consideration and a total of 2583 children were selected as "probable ADHD".

2.1.2. Phase II

The 2583 children with "probable ADHD" selected from Phase I were examined by a team of four clinicians comprising two psychiatrists (F.Y. and M.Ü.), one pediatrist (A.Y.) and one psychologist. The mother or father and teacher of the children were present during the course of examinations performed. Ultimately, from among the 2583 children examined, 618 (119 girls and 499 boys) were diagnosed as having ADHD (prevalence: 2.8%). Children who met DSM-IV (American Psychiatric Association, 1994) criteria for ADHD ($N = 628$) and who have not any other mental or medical illness ($N = 1965$, see below, Section 2.2) as assessed by clinical interview and physical examination were included in the study.

2.2. Patient selection, inclusion and exclusion criteria

The subjects ($N = 618$) were male and female outpatients, who fulfilled DSM-IV criteria for ADHD as determined by psychiatric evaluation. A patient was excluded if he or she had another axis I disorder and had any medical condition. Comorbid psychiatric and medical illnesses in the current and the past were assessed by medical anamnesis, physical examination and clinical interview with DSM-IV criteria. Patients including any comorbid illnesses were excluded. The comorbid illnesses ($N = 1965$) include: physical illnesses or taking any drug ($n = 1539$), enuresis ($n = 145$), conduct disorder ($n = 103$), mood and anxiety disorders ($n = 55$), mathematic disorders ($n = 47$), learning disorders ($n = 33$), encopresis ($n = 12$), communication disorders ($n = 11$), stuttering ($n = 10$), mental retardation ($n = 7$), Tourette disorder ($n = 3$). Other exclusion criteria were that taking any psychotropic and other medication, having intolerance to zinc,

having pathological findings in routine biochemical measurements, and taking medication that would affect the study results. Patients with history of clinically significant and currently relevant hematologic, renal, hepatic, gastrointestinal, endocrine, pulmonary, dermatologic, oncologic, or neurologic (including seizures or epilepsy) disease were excluded. Anyone with a history of chronic hepatitis and elevated liver enzymes as well as those known to be positive rheumatoid factor, was also excluded. Other reasons for exclusion included a history of hypersensitivity to any drug, use of psychostimulants, antipsychotic compounds and antidepressants at any time.

The parents of 618 patients were enlightened as to the scope of the study to be performed and a written consent was obtained. Furthermore, approval was also obtained for the study from the Ethic Committee of Medicine Faculty of KTU.

Patients who met the inclusion criteria ($n=618$) began a 1-week placebo lead-in phase to evaluate compliance with study procedures and to eliminate early placebo responders. At the conclusion of the lead-in phase, 218 patients also excluded from the study. Details of reasons for failure after the placebo lead-in phase are provided in patient flow diagram, Fig. 1.

2.3. Double blind treatment design

At the conclusion of the lead-in phase 400 (72 girls, 328 boys) eligible patients were then randomly assigned in a 1:1 ratio to receive oral zinc sulfate ($n=202$) or placebo ($n=198$) over a 12-week, double-blind treatment phase. Subjects who were noncompliant or had unresolved clinically significant abnormalities in laboratory or physical examination findings were not assigned to a condition.

A fixed-dose (150-mg) of zinc sulfate (produced by the Merck pharmaceutical company containing $ZnSO_4 \cdot 7H_20$, with approximately 40-mg zinc element) and placebo (sucrose, 150 mg) preparations were given for the participating patients. A daily dose of 150-mg was preferred; because of the maximum daily dose in zinc deficiency was 150 mg in the previous studies (Hemalatha et al., 1993). This dose was kept fixed throughout the course of the study. Study drug materials for both treatment groups were identical in appearance. The zinc sulfate and placebo preparations were orally administered for a period of 12 weeks by mixing into a breakfast drink such as fruit juice. Adherence was assessed by history.

The patients were evaluated at baseline (Visit 0), first week (Visit 1), 4th week (Visit 2), and 12th week (Visit 3). Routine biochemical and hematological tests, zinc and FFA levels were carried out twice during Visit 0 and Visit 3. All patients passed Visit 0 within a period of 4 weeks. Thus, a period of 12 weeks passed with the clearance of the first patient until the clearance of the last patient through the treatment.

2.4. Outcome variables

2.4.1. Attention Deficit Hyperactivity Disorder Scale (ADHDS)

The scale, which is developed by us, is clinician conducted and consisted of 46 questions. The ADHDS was developed from several sources: Clinical experiences with ADHD patients, analysis of literature on ADHD, and taking DSM-IV criteria into account. The questions are concerned with attention deficiency (question 1–15), hyperactivity (question 16–28), impulsivity (question 29–38), and impaired socialization (question 39–46). These 46 questions were weighted equally on a 0–2 scale (never = 0, sometimes = 1, and often = 2). Thus, the 46 question scores are summed to yield a global ADHDS score, which has a range of 0–92. The validity and reliability of the scale was assured and then used in the study. The ADHDS showed a good level of internal consistency (Cronbach's α=.86). The question–total correlation coefficients ranged from .96 to .52. Test–retest reliability of the ADHDS scale was performed by using t test and Pearson correlation analysis and results were good. The cutoff values of the subscales were obtained by using ROC analysis. Psychometric properties, validity and reliability of the test will be presented elsewhere (Bilici et al., unpublished data). The ADHDS was administered at baseline (Visit 0), and on weeks 1 (Visit 1), 4 (Visit 2), and 12 (Visit 3, or at study termination). ADHDS has four subscales:

- ADHDS, Attention Deficit Subscale (ADHDS-A)—There were 15 questions in this subscale. Those scoring 20 points or higher were evaluated with "attention deficiency"
- ADHDS, Hyperactivity Subscale (ADHDS-H)—This subscale consisted of 13 questions. Those scoring 16 points or higher were evaluated as having "hyperactivity"
- ADHDS, Impulsivity Subscale (ADHDS-I)—There were 10 questions in this subscale. Those scoring 12 points or higher were evaluated as having "impulsivity"
- ADHDS, Impaired Socialization Subscale (ADHDS-S)—There were eight questions in the subscale. Those scoring 12 points or higher were evaluated with "impaired socialization".

2.4.2. Turkish Adaptation of Conners Teacher Questionnaire (TACTQ)

The Turkish adapted and validated form of the 28-item TACTQ (Conners, 1990) was used in this study (Dereboy et al., 1997). TACTQ has three subscales:

- Ankara Conners Teacher Questionnaire, Attention Deficit Subscale (ACTQ-A)—Composed of six TACTQ items. Those scoring nine points or higher were evaluated with "attention deficiency"

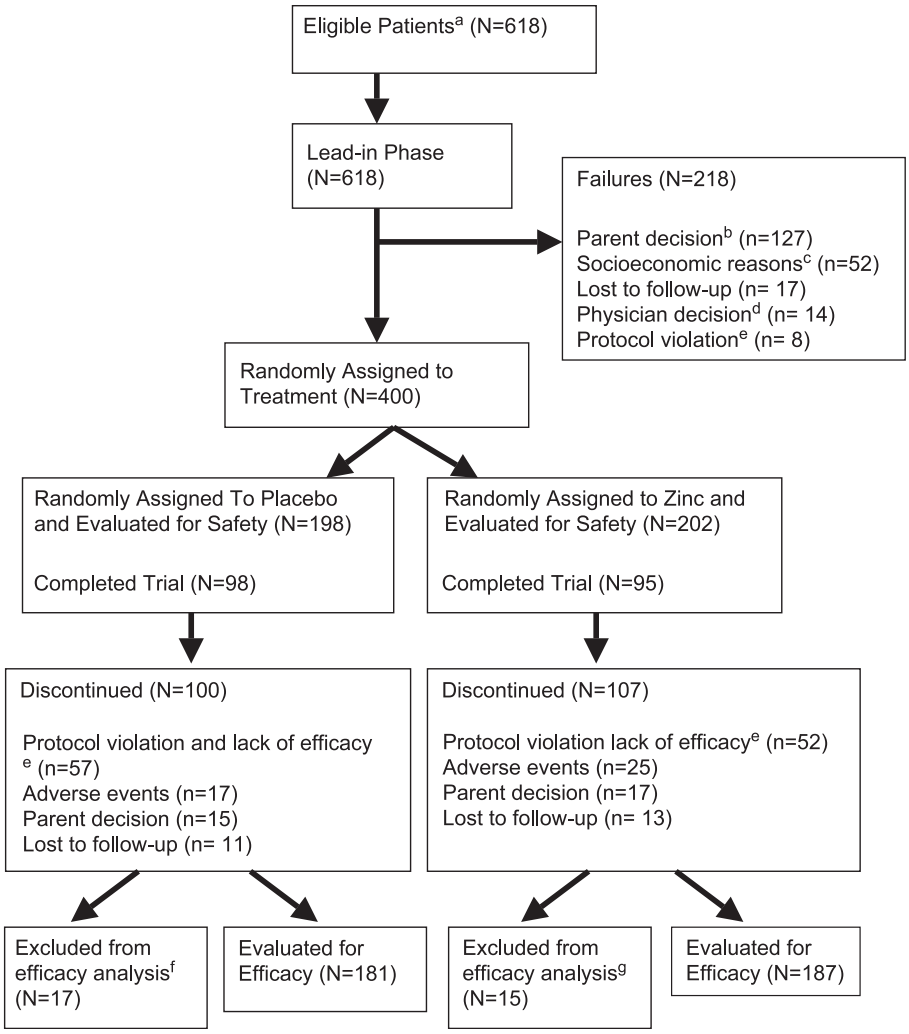

Fig. 1. Patient flow diagram. (a) Parents who signed an informed consent statement and had a baseline assessment. (b) Include parents who withdrew informed consent. (c) Include parents who have economic problems, not having health assurance, etc. (d) Include early placebo responders, patients who take any other drugs, and overdose. (e) This include late coming to visit and refusing blood draw or examination, etc. (f) This include poor data quality ($n=13$), no postbaseline data ($n=4$). (g) This include poor data quality ($n=12$), no postbaseline data ($n=3$).

- Ankara Conners Teacher Questionnaire, Hyperactivity Subscale (ACTQ-H)—Composed of six TACTQ items. Those scoring eight points or higher were evaluated as being "hyperactive/impulsive"
- Ankara Conners Teacher Questionnaire Conduct Subscale (ACTQ-C)—Composed of six TACTQ items. Those scoring eight points or higher were evaluated as having "Behavioural Disorder".

2.4.3. DuPaul Parent Ratings of ADHD (DuPaul)

This is a 14-item scale developed by DuPaul in 1990 (DuPaul, 1991). The intersection points for ADHD in the scale is different for males and females; for females the score of at least 8 of the 14 items, and for males the score of at least 10 of the 14 items should be a minimum of 2. The validity and reliability of the "DuPaul ADHD Evaluation Scale" for our country has not been determined. For this study, we converted the scale to Turkish and used it herein.

These scales, i.e., TACTQ ACTQ-A ACTQ-H ACTQ-C and DuPaul, were applied twice at the beginning of the study (Visit 0) and at the end (Visit 3). Full therapeutic response was defined as having less than 20 points on the ADHDS-A, less than 16 points on the ADHDS-H, and less than 12 points on the ADHDS-I and ADHDS. Safety and tolerability were assessed at each visit by open-ended questioning for adverse events and by monitoring of vital signs and laboratory data.

2.5. Laboratory methods

Under parental observation, following a 12-h period of fasting, intravenous blood sample of 5 ml was taken from the participating patient, between 08:00 and 09:00 a.m.

Blood sample was sent within an hour to the biochemistry laboratory and separated into serum after being put through a centrifuge for 15 min at 3000 rpm. To determine the levels of the fatty acids and the zinc, two aliquots were prepared and placed in a deep freezing unit at -20 °C. To maintain

standardization of results, all laboratory samples were measured in the same day.

2.5.1. Measurement of serum zinc level. The level of the serum zinc was measured in collaboration with the KTU Medical Faculty, Biochemistry Department, using the atomic absorption spectrometry (AAS) (GBC Scientific, Model 902). After being diluted with a 1/4 proportion of deionised water, the serum was placed in an AAS circumstance. The slit width was 0.5 nm, lamp flow was 5.0 mA, wavelength was 213.9 nm and air–acetylene gas mixture was implemented to designate the absorbances. The absorbances of standard zinc solutions (0, 10, 20, 40, 50 μg/dl) were measured and calibrated under the same conditions and plotted with the help of a computer to obtain the zinc values. The range of normal zinc serum levels of AAS method were between 70 and 150 μg/dl.

2.5.2. Measurement of the FFA levels. The FFA levels were measured using the colorimetric and enzymatic method and by using the Wako (code no. 994-75409E) commercial kit. The reactive used in our study includes acyl-CoA synthetase, CoA, ATP, 4-aminoantipirin, phosphate buffer and colour reactive A containing magnesium chloride; acyl-CoA oxidase and colour reactive B containing peroxidase along with 1.0 mEq/l standard solution containing linoleic acid during measurements, the serum sample and colour reactives were mixed well at a temperature of 37 °C and after an incubation period of around 10 min resultant mixture was subjected to absorbance reading at 550 nm wavelength (LKB model 4053 spectrophotometer). Calibration was done by a standard of 1.0 mEq and results were calculated using the formula: absorbance of the sample/absorbance of standard × 1 (mEq/l) (CV = 2.2%).

2.6. Statistical methods

The size of the study group was estimated on the basis of ADHDS parameters. A difference of five points on the ADHDS between treatment groups was deemed to the smallest clinically relevant difference in endpoint values. The standard deviation of the ADHDS was expected to be less than 18.2. On the basis of these parameters, a study group of 350 subjects (180 taking zinc and 170 taking placebo) was necessary to provide at least 80% power (α=.05, two-tailed) to detect such a mean difference.

Efficacy analyses were performed on intent-to-treat basis. All comparisons between the two treatment groups were analyzed for significance at the two-tailed .05 level. When appropriate, the last observation was carried forward to interpolate missing data.

Primary efficacy analyses were the differences from baseline to endpoint in mean ADHDS and TACTQ scores between the zinc and placebo groups. These variables were assessed by using analysis of covariance (ANCOVA) models with the baseline score used as a covariate.

Patients were identified as responders (decrease in ADHDS score $\geq$ 50% from baseline to specific time point) or nonresponders (decrease in ADHDS score $<$ 50% from baseline to specific time point) at each visit. The responder and nonresponder groups ratio were compared by using chi-square test.

Secondary efficacy analysis included the difference between the zinc and placebo groups in mean changes from baseline to endpoint in scores on the attention, hyperactivity, impulsivity and impaired socialization subscales of the ADHDS. Groups were compared with respect to these variables by using ANCOVA. The factors affecting the scale scores following treatment were evaluated by using regression analysis.

Descriptive statistics including Pearson correlation, chi-square and *t* test were applied to clinical and laboratory safety data for each within-group parameter. All numeric values were expressed as arithmetic mean ± S.D. These were then evaluated on the basis of tabular and graphic displays.

3. Results

3.1. Demographic characteristics

Of the 618 patients screened for study inclusion, 400 were randomly assigned to receive zinc (n = 202) or placebo (n = 198). Data from all 400 patients were used in the evaluation of the safety of zinc, and data from 95 and 98 patients in the respective treatment groups were used in the evaluation of efficacy (Fig. 1). Some sociodemographic and baseline clinical and laboratory characteristics of the patients are given in Table 1. At baseline, all patients had an ADHDS total score $\geq$ 65, and there were no statistically differences between the zinc and placebo groups with respect to any primary or secondary variable.

In the zinc group, but not in the placebo group, there was a significant increase in zinc levels during Visit 3 (140.6 ± 33.6) as compared to Visit 0 (88.8 ± 25.5) (t = 12.7, df = 96, P=.01). There was also a significant increase in FFA levels during Visit 3 (0.85 ± 0.38) as compared to Visit 0 (0.69 ± 0.39) (t = 4.1, df = 55, P =.03) in the zinc group.

Pearson correlation analysis showed there was no significant correlation between BMI or age and ADHDS subscale sores.

The median duration of treatment for patients assigned to receive zinc was 9.5 weeks. One hundred and twenty-eight of the 202 patients (63.3%) completed >4 weeks in randomized treatment. The median duration of treatment for patients assigned to receive placebo was 10.0 weeks. One hundred and thirty of the 198 patients (65.6%) completed >4 weeks in randomized treatment. Of the 400 patients, 193 (48.2%) completed 12 weeks of treatment. Of the 400 patients, 207 (51.8%) discontinued at the 12-week trial. Of those remaining outside the scope of

Table 1
Baseline socio-demographic, clinical and laboratory characteristics of the patients

	Zinc ($n=202$)	Placebo ($n=198$)	t or χ^2	df	P
Age	9.4 ± 1.5	9.7 ± 1.8	0.14	191	.56
BMI (kg/m^2)	13.2 ± 1.7	12.9 ± 2.9	0.42	191	.51
Sex (F/M)	40/162	32/166	0.21	2	.22
Duration of Illness (year)	6.2 ± 3.4	6.7 ± 4.2	0.17	191	.64
ADHDS (total)	75.8 ± 11.3	74.6 ± 12.7	2.37	191	.74
ADHDS-A subscale	26.6 ± 6.5	26.6 ± 7.0	1.03	191	.78
ADHDS-H subscale	23.8 ± 7.9	21.8 ± 7.1	1.45	191	.84
ADHDS-I subscale	14.5 ± 5.3	13.9 ± 5.2	1.27	100	.88
ADHDS-S subscale	12.5 ± 3.5	12.4 ± 3.5	1.75	191	.89
ACTQ-A subscale	11.1 ± 5.1	10.2 ± 3.4	1.83	191	.88
ACTQ-H subscale	11.6 ± 3.5	10.5 ± 3.2	2.07	191	.89
ACTQ-C subscale	9.6 ± 4.0	9.8 ± 6.1	2.06	191	.73
DuPaul	19.6 ± 7.3	20.2 ± 7.4	1.98	191	.52
Visit 0 zinc level (μg/dl)[a]	88.8 ± 25.5	91.4 ± 23.2	0.72	191	.43
Visit 0 FFA level (mEq/l)[a]	0.69 ± 0.39	0.77 ± 0.41	1.3	100	.48

[a] Normal references ranges of zinc (77–107 μg/dl) and FFA (0.2–1.1).

the study 107 were from the zinc group (52.9%), 100 were from the placebo group (50.05%) and there is no statistical difference in the drop out rate of the two groups ($\chi^2=0.74$, $df=2$, $P>.05$).

3.2. Clinical response

Treatment with zinc sulfate improved hyperactivity, impulsivity and impaired socialization symptoms of ADHD in primary and secondary efficacy variables. On week 4, the zinc-treated patients demonstrated significant improvement in the total ADHDS score, compared with placebo-treated patients. By week 12, the zinc-treated patients showed significant improvement in ADHDS-H, ADHDS-I, ADHDS-S, ACTQ-H and ACTQ-C subscales compared with placebo recipients.

On the 46-item ADHDS, zinc-treated patients achieved a reduction in mean score of 25.4 ± 9.7 points from baseline to endpoint. Placebo-treated patients experienced a decrease of 12.7 ± 5.4 over the same period. The difference between groups was significant ($F=11.7$, $df=1,191$, $P=.002$). A significant difference between zinc and placebo groups in mean change in the ADHDS score was evident by the 4th week of treatment and was maintained throughout the 12-week trial (Fig. 2).

The mean reductions in scores on the ADHDS attention deficit subscale (ADHDS-A) were 0.1 ± 0.4 for the zinc group and 0.3 ± 0.7 for the placebo group ($F=1.2$, $df=1,187$, $P=.89$). The respective group decreases in score on the hyperactivity (ADHDS-H) ($F=7.2$, $df=1,187$, $P=.01$), impulsivity (ADHDS-I) ($F=5.8$, $df=1,187$, $P=.03$), and impaired socialization (ADHDS-S) ($F=5.9$, $df=1,187$, $P=.03$).

At endpoint ACTQ-H subscale scores were reduced from baseline by 3.5 ± 1.4 zinc-treated patients and by 0.3 ± 0.2 placebo-treated patients ($F=7.2$, $df=1,187$, $P=.03$). Similarly, the mean reductions in scores on the ACTQ-C subscale were 2.4 ± 1.4 for the zinc group and 0.8 ± 0.3 for the placebo group ($F=5.2$, $df=1,187$, $P=.04$). However, the mean changes in scores on the ACTQ-A and DuPaul between groups were not statistically different.

Table 2 shows the cutoff values of the ADHDS subscales in the groups, before and after 12-week treatment periods. As seen in the Table 2, rates of recovered patients according to post treatment cutoff values of the subscales of ADHDS-H, ADHDS-I and ADHDS-S, and the full therapeutic response rates in the zinc group were significantly higher than those of the placebo group. The full therapeutic response rates of the male and female patients (27.7% and 29.7%) in the zinc group were not significantly different ($\chi^2=0.15$, $df=2$, $P>.05$).

Regression analysis showed factors affecting the response of patients taking zinc, which are changes between Visit 0 and Visit 3 scores of ADHD-H, ADHD-I and ADHD-S, were significantly affected by age, body mass index (BMI), and Visit 0 FFA and zinc levels. Details are given in Table 3.

3.3. Tolerability

Discontinuation of treatment occurred in 52.9% (107 of 202) of patients assigned to receive zinc and 50.5% (100 of 198) assigned to receive placebo. Those withdrawing because of protocol violation and lack of efficacy represented 25.7% (52 of 202) and 28.7% (57 of 198), respectively, of the two groups; those withdrawing because of adverse events 12.3% (25 of 202) and 8.5% (17 of 198); and those withdrawing for other reasons 14.8% (30 of 202) and 13.1% (26 of 198). The most frequently reported adverse events are listed in Table 4. Metallic taste in the mouth was the most common reason cited, occurring in 58 patients (50 taking zinc sulphate, and 8 taking placebo). As seen in the Table 4, there were no significant differences, except metallic taste in the mouth,

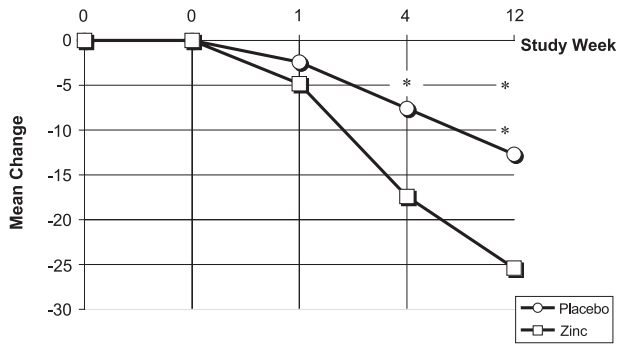

Fig. 2. Mean change in ADHDS total score from baseline in patients with ADHD in a 12-week placebo controlled trial evaluating the efficacy of zinc for the treatment of ADHD. (Last observation carried forward.) * Significant difference between zinc treated patients and placebo treated patients ($P=.01$, F test). ** Significant difference between zinc-treated patients and placebo treated patients ($P=.002$, F test).

Table 2
The rates of recovered patients according to cutoff values of the ADHDS subscales

	Zinc, *n* (%)	Placebo, *n* (%)	χ^2	*df*	*P*
ADHDS-A < 20 (Visit 0)	27 (28.7)	32 (32.6)	0.89	2	.43
ADHDS-A < 20 (Visit 3)	34 (35.8)[a]	30 (30.6)	1.29	2	.38
ADHDS-H < 16 (Visit 0)	25 (26.3)	24 (24.4)	1.91	2	.31
ADHDS-H < 16 (Visit 3)	35 (36.8)[a]	21 (21.4)	4.84	2	.02
ADHDS-I < 12 (Visit 0)	23 (24.2)	28 (28.5)	1.85	2	.41
ADHDS-I < 12 (Visit 3)	39 (41.0)[a]	26 (26.5)	3.94	2	.03
ADHDS-S < 12 (Visit 0)	24 (25.2)	22 (22.4)	1.52	2	.45
ADHDS-S < 12 (Visit 3)	34 (35.8)[a]	19 (19.3)	4.96	2	.02
Full Therapeutic Response	27 (28.7)	20 (20.4)	3.04	2	.04

[a] In the zinc group, rates of recovered patients according to cutoff values of the ADHDS subscales were significantly higher in Visit 3 period than the rates of Visit 0 period (chi square test, all *P* values are < 0.05).

in reasons for discontinuation in treatment among the groups. Clinically significant changes in laboratory values were not found patients in either group. No change in median values for heart rate were observed from baseline to endpoint in either group. No significant change in weight was determined with either treatment.

4. Discussion

The most widely used medications for ADHD are the psychostimulants methylphenidate and amphetamine (Popper, 2000). There is, however, considerable interest in alternative, nonstimulant therapies, because some patients respond inadequately to stimulants or are unable to tolerate them. In addition, some physicians are unenthusiastic to use stimulants because of concerns about misuse in a population at increased risk for substance abuse (Wilens and Biederman, 1992).

Table 3
Factors affecting the change in scores of ADHD-H, ADHD-I and ADHD-S in the zinc group

	B	S.E.	Beta	*t*	*P*
ADHD-H					
Constant	1.54	0.52		2.79	.03
Age	2.02	0.47	0.93	2.41	.04
BMI	3.03	0.61	0.95	2.61	.03
Visit 0 Zn	− 0.42	0.11	1.09	3.21	.03
Visit 0 FFA	− 5.14	1.23	1.07	2.87	.03
ADHD-I					
Constant	1.49	0.57		2.76	.03
Age	3.12	0.71	0.91	2.47	.04
BMI	2.72	0.84	0.90	2.18	.04
Visit 0 Zn	− 0.51	0.63	1.87	3.19	.03
Visit 0 FFA	− 1.47	1.27	1.79	2.79	.03
ADHD-S					
Constant	1. 51	0.77		2.69	.03
Age	3.08	0.79	0.83	2.56	.04
BMI	2.04	0.88	0.89	2.82	.04
Visit 0 Zn	− 0.54	0.60	1.95	3.52	.03
Visit 0 FFA	− 2.86	1.28	1.92	2.61	.03

Table 4
Adverse effects of treatment in the groups

	Zinc, *n* (%)	Placebo, *n* (%)	χ^2	*df*	*P*
Metallic taste	50 (52.6)	8 (8.1)	4.18	2	.01
Nausea	8 (3.9)	7 (3.5)	0.78	2	.55
Vomiting	5 (2.4)	4 (2.0)	0.92	2	.45
Abdominal pain	3 (1.4)	2 (1.0)	0.79	2	.59
Diarrhea	1 (0.4)	1 (0.5)	0.95	2	.51
Acute Infection	5 (2.4)	7 (3.5)	0.34	2	.65
Undergoing medical treatment	10 (4.9)	8 (4)	0.52	2	.41

In this study, zinc was effective and well tolerated in patients with ADHD. Compared with placebo, zinc treatment produced sustained improvement from baseline in the outcome measures of the ADHDS scale scores.

4.1. Mechanism of action

In this study, the treatment of zinc sulfate showed significantly improvement in hyperactivity, impulsivity and socialization scores, in ADHD patients; however, there was no positive effect on attention deficiency score. It is still unclear as to the mechanism of the positive effect of zinc treatment on hyperactivity, impulsivity and socialization; however, it is possible to find various speculations in this regard. It is known that zinc is necessary for many metal–enzyme complexes (Toren et al., 1996), many of them located in the nervous system, and that it contributes to construction and function of the brain (Black, 1998). In addition, zinc is essential for conversion of dietary pyridoxine to its active form, pyridoxal phosphate, and pyridoxine is necessary for conversion of tryptophan to serotonin. It is known that there is a close relation between serotonin and ADHD.

In an uncontrolled study, it was shown that serotonin reuptake inhibitors were effective on ADHD; other studies show that the level of blood serotonin in ADHD is low (Arnold and Jensen, 1998). In terms of serotonergic functions, aggressive and nonaggressive ADHD children are found to be different, with an increase in prolactine response to 5-HT agonist, fenfluramine, in aggressive ones; however, there are no differences in the measurement of the peripheral neurotransmitter functions (Halperin et al., 1998). These findings suggest that zinc might affect some ADHD symptoms, such as impulsivity through increasing of serotonergic functions.

Furthermore, zinc is also basic for production and modulation of melatonin, which helps regulate dopamine function (Chen et al., 1999), supposed to be an important factor in ADHD and its treatment. Indeed, some reports hypothesized that parasympathomimetic stimulants work in ADHD partly via effects on melatonin (Sandyk, 1990). Therefore, it is suggested that the supplementation of zinc may remove the reduction in melatonin and serotonin

synthesis and consequently improve aggressive behaviour and impulsivity.

The relative lack of fatty acids in modern Western diets has been highlighted as a potential major health issue and, recently, attention has been focusing on their potential role in a wide range of neurodevelopmental and psychiatric conditions, including ADHD (Richardson and Puri, 2000). Blood biochemical evidence has suggested that a relative deficiency of certain fatty acids may contribute to some of the behavioral and learning problems central to ADHD (Alexandra and Basant, 2002). On the other hand, zinc is also involved in essential fatty acid (EFA) metabolism in a number of ways (Bekaroglu et al., 1996; Prows and Schroeder, 1997). EFA is a substrate for delta-6-desaturase (Eder and Kirchgessner, 1996) and modulates cyclooxygenase activity (Sakuma et al., 1996). Cyclooxygenase is essential for production of cell-regulating prostaglandins and thromboxanes from EFA precursors. In turn, zinc absorption is facilitated by prostaglandins (Song and Adham, 1980). EFAs and their metabolites are also involved in dopamine and norepinephrine metabolism (Molderings et al., 1992; Racke et al., 1992) and vice versa (Nadasy et al., 1992). Bekaroglu et al. (1996) reported a significant correlation between serum zinc and serum FFA levels in patients with ADHD, who had significantly lower FFAs and zinc than healthy control subjects. Zinc may exert a protective effect against the oxidative risk of high n-3 fatty acid intake (Villet et al., 1997).

In light of above knowledge, it can be suggested that there is a synergism of zinc and EFAs in regulating dopamine, norepinephrine, and possibly serotonin activity, with implications for treatment of ADHD. According to another study, zinc-deficient ADHD patients respond less to dextroamphetamine in comparison to those who did not have a deficiency (Arnold and Votolato, 1990). Arnold et al. (1990) reported a significant correlation of baseline hair zinc with placebo-controlled d-amphetamine response in ADHD, but did not investigate relationships with fatty acids or use other tissue measures of zinc. Later report explores further the relationship of zinc nutrition, essential fatty acid effects, and stimulant effects in treatment of ADHD, utilizing three measures of zinc nutritional status. Our findings and these results showed that fatty acids are important in brain development and function, and the above findings raise the possibility that dietary zinc supplementation with a combination fatty acids might be beneficial for some children with ADHD.

4.2. The role of FFA

In our study, demonstrated that posttreatment serum zinc and FFA levels significantly increased in the zinc group. This was an expected result in the group of patients taking zinc. Considering that the zinc and FFA levels increased in patients taking zinc sulfate, and did not do so in the placebo group, it may be considered as an indication that the patients were regularly having zinc sulfate.

In ADHD patients, zinc deficiency has been implicated in the metabolism of dietary fatty acids to form prostaglandin (McGee et al., 1990). This finding may be explained in such a way that zinc activates the formation of certain lipid FFA types through certain desaturase enzymes or some other mechanism. Considering that there was a significant increase in the levels of FFA in our studies of the patients taking zinc, this biochemical relationship is thus supported. Further, taking account of the data accumulated, it may well be considered that some ADHD symptoms may have been improved through the rising of FFA levels due to zinc intake.

4.3. Therapeutic response

When taking into account the assessment of the global ADHDS score and subscales cutoff score of the ADHDS, improvement of hyperactivity, impulsivity and socialization rates in the zinc group were significantly high in comparison to the placebo group. From these findings, it was found that only some of the ADHD patients returned to normal functioning following the zinc treatment. It is for this reason, although zinc does not affect all symptoms of ADHD, it may still be implemented as a supportive agent in the treatment of ADHD. However, when examining Table 2, it was observed that the rate of patients returning to normal functioning according to the ADHD subscales in the zinc group ranged between 25% and 41%, whereas those ratios were 19% to 32% in the placebo group. In a similar manner, the full therapeutic response ratio in the zinc group was 28.7% while it was observed to be 20.4% in the placebo group. Although this ratio is significantly high in the zinc group, it may still be noted that around 70% of them did not completely return to the normal functioning. These findings suggest that treatment of zinc by itself in ADHD does not constitute a full therapeutic response; therefore, it is thought that it would need to be applied along with a supplementary medication.

In ADHD patients taking zinc, it was determined that older age and high BMI values, along with low pretreatment zinc and FFA values, led to a significant improvement in hyperactivity, impulsivity and social inappropriateness (see Table 3). In the light of these observances, it may be suggested that older age and higher BMI in children suffering from ADHD could benefit more from the improvement in hyperactivity, impulsivity and socialization after zinc treatment.

Arnold et al. (2000) to explore the relationship of zinc nutrition to essential fatty acid supplement and stimulant effects in treatment of ADHD, they reanalyzed data from an 18-subject double-blind, placebo-controlled crossover treatment comparison of d-amphetamine and ephamol (evening primrose oil, rich in gamma-linolenic acid). This study suggests that zinc nutrition may be important for treatment of ADHD even by pharmacotherapy; and if ephamol benefits ADHD, it likely does so by improving or compensating for

borderline zinc nutrition. These data are in accordance with our results.

4.4. Adverse events

No significant difference was found in the rates and reasons for discontinuation in treatment in the zinc and placebo groups (see Table 4). This finding suggested that the tolerability of zinc and placebo was similar; and that maintaining a fixed daily dose of the zinc was an important factor in increasing tolerability. The rate of withdrawal from the treatment was found to be 52.9% and 50.5% in the zinc and placebo groups, respectively; and these rates were not significantly different. This finding suggests that independent to the treatment, withdrawal from the treatment was a high ratio in ADHD patients. Metallic taste in the mouth was the most frequently cited reason, occurring in 58 patients having ADHD (50 taking zinc sulphate, and 8 taking placebo).

4.5. Limitations

The most important limitations of this study were the maintenance of a fixed zinc dosage, short duration of treatment, and the lack of establishment of a control patient group to take stimulant drug, amphetamine. For this reason, implementing a study after removal of the aforementioned limitations could well provide interesting results. Furthermore, feeding habits of the patients participating in the treatment was a variable that was not kept under control; possible effects on the results were neglected. This neglect should be noted as a limitation in the study.

The other important limitation was that other detailed psychological tests measuring cognitive deficit or questionnaire like BRIEF are more sensitive to changes in ADHD symptoms with medication were not be used by the authors; however, the reliability and validity of those tests have not been conducted in Turkey. Thus, the authors had to develop the new test (ADHDS) used in the study.

5. Conclusion

In conclusion, this double-blind, placebo-controlled study demonstrated that zinc is effective and well tolerated in patients with ADHD. In the treatment of ADHD zinc sulfate displayed positive effects on the symptoms of ADHD, although these were somewhat limited. For this reason, it is suggested that the treatment of zinc in ADHD would be more beneficial as a supplementary medication rather than by itself. The response of zinc treatment would be augmented in cases that have low pretreatment serum FFA and zinc levels, their ages are older and BMIs are higher. Although maintaining the daily zinc sulfate dose at 150 mg increased tolerability, in return, the rate of response to the treatment could well have fallen. So, further investigations and different doses of zinc are required to replicate these findings in patients with ADHD.

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