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Serum Levels of Zinc and Copper in Cases of Juvenile Idiopathic Arthritis

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Authors' contributions

This work was carried out in collaboration among all authors. Author HSEDT designed the study protocol, supervised the whole work and reviewed the manuscript. Author ESS collected data and managed the literature searches and the analysis of the study. Author AIA performed the laboratory work. Author MFM designed the study protocol, supervised the whole work and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Juvenile idiopathic arthritis (JIA) represents a heterogeneous group of autoimmune diseases that arises before the age of 16 years and lasts more than 6 months. During acute inflammation of the disease, serum copper concentration increases and zinc decreases, that could point to the possible pharmacological properties of these trace elements.
Aim: To measure the serum level of zinc and copper in patients with juvenile idiopathic arthritis (JIA) with different subtypes and correlate the levels of zinc and copper with the disease activity.
Methods: This cross-sectional study was done on 40 patients already diagnosed clinically with JIA; patients were followed-up at the Rheumatology Outpatient Clinic, Children's Hospital, Cairo University.
Results: Out of forty patients, 16 were males (40%) and 24 were females (60%) with a male to female ratio (M: F) of 1:1.5. Out of the forty patients 17 were in activity and 23 were without activity. Thirty age and sex matched controls were included for comparison. Serum copper level was

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significantly higher in patients with JIA than those of the controls (P= 0.017) while there were no significant difference in serum level of zinc between JIA patients and that of the controls. **Conclusion:** Alteration of serum copper and zinc probably is a defense response against JIA; increased copper may be due to inflammation associated, these elements could serve as biomarkers for the disease activity.

Keywords: Juvenile idiopathic arthritis; zinc; copper.

1. INTRODUCTION

Juvenile idiopathic arthritis (JIA), a heterogeneous group of autoimmune diseases, that arises before the age of 16 years and lasts more than 6 months [1]. The incidence and the prevalence of the disease vary from 2 to 20 and from 16 to 150 per 100,000 respectively; JIA is an important cause of short and long term disability [2,3]. Clinical presentation is variable; children with polyarticular or systemic onset JIA usually present with anorexia, weight loss, fatigue, and growth failure; however, these manifestations are not common in oligoarticular JIA [4]. Morning stiffness is a common manifestation of joint inflammation, but this could be infrequently described in young children; and instead they usually refuse to use the affected joint and do not complain of joint pain [5]. Copper and zinc are essential nutrients; they are constituents of the superoxide-dismutase enzyme which has intracellular antioxidant functions [6]. Önal et al. in 2011 found that the copper level was significantly higher and zinc was lower in JIA patients relative to controls, they also noted that treatment with methotrexate resulted in elevation of serum zinc levels; on the other hand, treatment with salazopyrin, corticosteroids, chloroquine, and non-steroidal anti-inflammatory drugs did not change the levels of any of the elements studied [7]. The aim of this study was to measure serum level of zinc and copper in JIA patients with different subtypes, relate zinc and copper levels to the disease activity and measure these levels of zinc and copper with different type of treatment.

2. MATERIALS AND METHODS

This cross sectional study included 40 patients already diagnosed clinically with JIA; who were attending Rheumatology Outpatient Clinic, Children's Hospital, Cairo University for follow-up. Patients with JIA started below the age of 16 years of more than one year duration were included in the study. However, patients with other rheumatologic diseases, and patients with JIA with less than one year disease duration were excluded. Thirty age and sex matched controls were included for comparison.

All included patients were fully assessed by history taking including; duration of disease, the disease activity, the number of inflamed joints, the type and duration of therapy, and the type of diet especially diet rich in zinc and copper (e.g. vegetables and fruits), and thorough clinical examination stressing on the anthropometric measurements (weight, and height). Patients records were reviewed especially for complete blood picture (CBC), erythrocyte sedimentation rate (ESR), kidney functions (serum urea and creatinine), aspartate trasaminase (AST), alanine trasaminase (AST), and urine analysis. The results of eye examination will be also reviewed in some cases. Serum zinc and copper level were measured to all patients and controls using atomic absorption spectrophotometry.

Patients were classified according to American College of Rheumatology (ACR) [8] criteria into: group 1 (JIA in remission) and group 2 (JIA in activity). Patients were identified as being in activity if they recently complained of arthralgia and/or arthritis and/or fever.

Preliminary criteria for inactive disease and clinical remission of JIA:

A) Inactive disease: 1) No joints with active arthritis* 2) No fever, rash, serositis, splenomegaly, or generalized lymphadenopathy, (3) no active uveitis, 4) normal ESR and CRP, 5) Global physical assessment indicates no activity.
B) Clinical remission: 1) Remission on medication: inactive disease criteria for a minimum of 6 consecutive months while on medication, 2) Remission off medication: inactive disease criteria for a minimum of 12 consecutive months while all medications for arthritis are off.

Note: As defined by ACR: A joint with swelling not due to bony enlargement, or if not present, limitation of motion with either pain on motion and/or tenderness.

2.1 Laboratory Tests

Two milliliters of blood were withdrawn from each patient and control, transferred into plain tubes and were let to stand until clotting. Clotted blood was centrifuged, separated, and serum transferred into aliquots and stored at -20ºC until measurement. Copper and zinc were determined from serum using atomic absorption spectrometry technique. Instrumental, gas-flow settings, aspiration rates, wave lengths and flame were adjusted precisely, to optimize signal for each of zinc and copper. This was done on the A analyst spectrophotometer from Perkin Elmer (940 Winter Street, Waltham, Massachusetts, 02451 USA).

Consents were obtained from every parent/surrogate of patients. The Scientific Research Committee of the Pediatrics Department, Cairo University approved the study protocol. Data confidentiality was preserved. Data were statistically described in terms of mean±SD, median and range, or frequencies and percentages when appropriate. Comparison of numerical variables was done using Mann Whitney test. For comparing categorical data, cross tab and Chi square test was performed. P-values less than 0.05 were considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows.

3. RESULTS

Out of forty JIA patients, 16 were males (40%) and 24 were females (60%) with a male to female ratio (M: F) of 1:1.5. Patients were classified according to disease activity into: group 1 (JIA in remission) and group 2 (JIA in activity). Patients were identified as being in activity if they recently complained of arthralgia and/or arthritis and/or fever. Group 1 included 23/40 patients (57.5%) of whom 9 (39.1%) were males and 14 (60.9%) were females (M:F=1:1.5) while group 2 included 17/40 patients (42.5%) of whom 7 (41.2%) were males and 10 were females (58.8) (M:F=1:1.4). The mean age of JIA in remission was 10.28±4.41 years (range, 3-22 years), while the mean age of JIA in activity was 9.65±4.11 years (range, 3.6-17 years), while; such difference was not statistically significant (p=0.649).

The mean age of onset of all study population was 5.44±3.65 ranging from 1-15 years (median, 4 years), while the mean duration of illness was 4.64±2.89 years ranging from 0.5–13 years (median, 5 years). There is no statistical difference between the two groups regarding age, age of onset, duration of illness, consanguinity and family history (Table 1).

Regarding eye examination, in JIA in remission 20 out of 23 patients (87%) showed normal eye examination, 2 patients (8.7%) had uveitis, one patient (4.3%) had retinitis, while in JIA in activity, all patients (100%) showed normal eye examination. There was no statistical significance between two groups (p=0.302).

Regarding abdominal examination, in JIA in remission, 3 patients (13%) had hepatosplenomegaly while in JIA in activity; only one patient (5.9%) had hepatosplenomegaly; there was no statistical significance between groups (p=0.455).

Our JIA patients were compared with thirty age and sex matched healthy controls. Comparing serum zinc and copper between JIA cases and controls, the mean serum zinc in cases was 79.88±38.17 microgm/dl while in controls it was 69.96±11.40 microgm/dl, with no statistical significance between cases and controls (p=0.17). However, serum copper level was statistically higher in JIA cases than that of the controls (p=0.017) (Table 2).

In JIA in activity the mean serum zinc and copper levels were statistically higher than controls (p=0.022 and 0.018 respectively) (Table 4). In JIA in remission the mean serum zinc was 72.47±32.30 microgm/dl while in controls it was 69.96±11.40 microgm/dl, with no statistical significance between two groups (p=0.693), on the other hand, the mean serum copper was 69.29±21.34 microgm/dl, that was statistically higher than that of the controls 58.14±12.78 microgm/dl, (p=0.021) (Table 3).

Comparing serum level of copper in JIA in activity and JIA in remission and control groups revealed that serum level of copper was significantly higher in JIA with activity than other two groups (p=0.036). However there is no significant difference between three groups regarding serum zinc level (p=0.073) (Table 4).

Fig. 1 illustrated the percent of patients receiving medications in JIA with activity and JIA in remission. There is no statistical significance between two groups regarding medications given (Fig. 1) (p >0.05).

Table 1. Demographic features of studied group

Item	JIA in remission (n=23)	JIA in activity (n=17)	p-value
Age (years) (mean±SD)	10.28±4.41 (range, 3-22)	9.65±4.11 (range, 3.6-17)	0.65
Age of onset (years) (mean±SD)	5.38±3.62 (range, 1-15)	5.51±3.81 (1.6-15)	0.98
Duration (years) mean±SD	5±3.24 years (range, 0.5-13)	4.15±2.35 (range, 1-8)	0.65
Consanguinity N (%)	9 (39.1%)	7 (41.2%)	0.89
Family history N (%)	1 (4.3%)	0 (0)	0.38

**P-value less than 0.05 is considered statistically significant*

Table 2. Serum zinc and copper levels in JIA cases and controls

	Cases (n=40)	Control (n=30)	p-value
Zinc (microgm/dl) (mean±SD)	79.88±38.17 (median, 70) (range, 45-200)	69.96±11.40 (median, 70.5) (range, 50-98)	0.17
Copper (microgm/dl) (mean±SD)	70.19±22.47 (median, 67) (range, 30-115)	58.14±12.78 (median, 58.05) (range, 40.5-87)	0.01*

**P-value less than 0.05 is considered statistically significant*

Table 3. Comparing serum zinc and copper levels in JIA in activity and controls, JIA in remission and controls, and JIA in activity and JIA in remission

	JIA in activity (n=17)	Control (n=30)	p-value
Zinc (microgm/dl) (mean±SD)	89.91±43.93 (median, 82) (range, 46-200)	69.96±11.40 (median, 70.5) (range, 50-98)	0.022*
Copper (microgm/dl) (mean±SD)	71.41±24.53 (median, 60) (range, 40-115)	58.14±12.78 (median, 58.05) (range, 40.5-87)	0.018*
	JIA in remission (n=23)	**Control (n=30)**	**p-value**
Zinc (microgm/dl) (mean±SD)	72.47±32.30 (median, 66) (range, 45-200)	69.96±11.40 (median, 70.5) (range, 50-98)	0.69
Copper (microgm/dl) (mean±SD)	69.29±21.34 (median, 73) (range, 30-115)	58.14±12.78 (median, 58.05) (range, 40.5-87)	0.021*
	JIA in remission (n=23)	**JIA in activity (n=17)**	**p-value**
Zinc (microgm/dl) (mean±SD)	72.47±32.30 (median, 66) (range, 45-200)	89.91±43.93 (median, 82) (range, 46-200)	0.15
Copper (microgm/dl) (mean±SD)	69.29±21.34 (median, 73) (range, 30-115)	71.41±24.53 (median, 60) (range, 40-115)	0.77

**P-value less than 0.05 is considered statistically significant*

Serum levels of zinc and copper in JIA patients in relation to different medications were illustrated in Table 5. It was observed that JIA patients on hydroquine had the lowest mean±SD serum zinc and copper levels [68.87±19.54 microgm/dl (median 65, range 46-100), and 62.40±17.15 microgm/dl (median 60, range 40-95), respectively]. However, JIA patients on prednisone drugs had the highest mean±SD serum zinc and copper levels [88.82±44.31 microgm/dl (median 80, range 50-200), and 72.09±23.31 microgm/dl (median 69, range 32-115), respectively] in comparison to patients receiving other medications. It is important to note that our patients were receiving more than one drug.

Table 4. JIA in activity, JIA in remission and controls regarding serum zinc and copper levels

	JIA in activity (n=17)	JIA in remission (n=23)	Control(n=30)	p-value
Zinc (microgm/dl) (mean±SD)	89.91±43.93 (range, 46-200)	72.47±32.30 (range, 45-200)	69.96±11.40 (range, 50-98)	0.073
Copper (microgm/dl) (mean±SD)	71.41± 24.53 (range, 40-115)	69.29 ±21.34 (range, 30-115)	58.14±12.78 (range, 40.5-87)	0.036*

**P-value less than 0.05 is considered statistically significant*

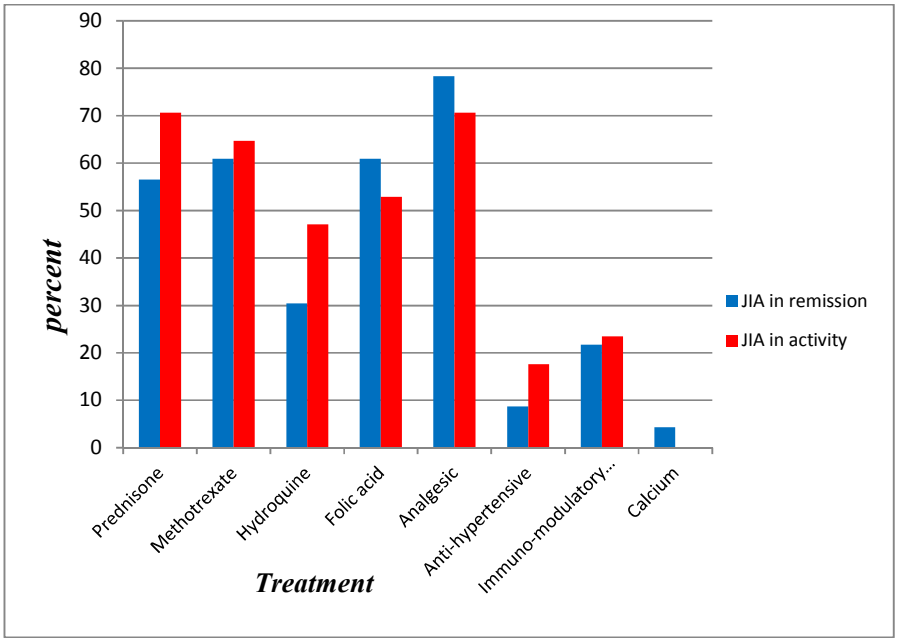

Fig. 1. Medications given to JIA patients in remission and in activity
Note: More than one patient were receiving more than one medication

Comparing treatment of JIA patients with serum levels of Zinc and Copper revealed that serum levels of zinc were statistically higher in patients treated by prednisone than those not treated by prednisone (p=0.03). No statistical significance could be detected regarding other drugs (Table 6).

4. DISCUSSION

Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disease in childhood of unknown etiology; it is a leading cause of short- and long-term disability. The disease is lasting at least 6 weeks and with an onset before the age of 16 years, it includes different subgroups that predominantly presents with arthritis. [2,9]. The importance of trace elements e.g., zinc and copper in chronic inflammatory arthritis is related to their role in different metabolic processes in articular tissues and in the functions of the immune system [10].

Our study included 40 patients already diagnosed clinically with JIA; who were attending Rheumatology Outpatient Clinic, Children's Hospital, Cairo University for follow-up with a male to female ratio of 1:1.5. Increased numbers of female patients can be related to the more possibility of autoimmune disease in females [11].

The mean age of onset of all study population was 5.44±3.65 ranging from 1-15 years (median, 4 years), comparable with ages described by other studies [12,13,14].

JIA can be classified into 3 major subgroups after 6 months of the onset of the disease into oligoarticular (affecting ≤4 joints), polyarticular (affecting ≥5 joints) and systemic onset [15]. Our patients were divided into oligoarticular 19 cases

(47.5%), polyarticular 17 cases (45.5%), and systemic onset 4 cases (10%) which was consistent with Quartier and Prieu (2007) and Danner et al., (2006), who stated that oligoarticular JIA was the most frequent type [16,17].

In our study, no statistical significance difference between cases and controls regarding serum zinc was noted (p=0.17); that was in concordance with Silverio et al., 2003 and Yazar et al., 2005. [6,18], but inconsistent with Ala et al., 2009 [10] and Onal et al., 2011 [7] who found a significant lower serum Zinc level in patients than controls and stated that the decreased in serum zinc probably related to the defense response of organism and are mediated by inflammatory like substances. In our study, serum copper level was statistically higher in JIA cases than that of the controls (p=0.01). Similar results were noted by other studies [6,7,18] but in consistent to Ala etal., 2009, who reported that serum copper levels of JIA patients did not change in comparison to controls and related this the various nutrition habits of people in different geographic areas; an serum copper and zinc levels could be affected by trace elements of diet [10].

In JIA patients, the consumption of zinc in inflammatory tissue and liver may be related to decreased serum zinc level, while, increased serum copper level is related to the cytokine related inflammatory response [19].

Regarding serum zinc and copper levels in JIA patients in activity and in remission; in our study, despite serum zinc and copper levels were higher in JIA patients in activity in comparison to JIA patients in remission, such differences were of no statistical significance (p=015, and 0.77 respectively). Regarding serum zinc, our results

Table 5. JIA cases and controls regarding serum zinc and copper levels in relation to different medications

Medication	Total JIA cases*(n=40)		
	N (%)	Zinc microgm/dl (mean±SD)	Copper microgm/dl (mean±SD)
Prednisone	25 (62.5%)	88.82±44.31 (median, 80) (range, 50-200)	72.09±23.31 (median, 69) (range, 32-115)
Methotrexate	25 (62.5%)	71.86±16.41 (median, 70) (range, 45-100)	68.22±21.19 (median, 65) (range, 30-97)
Hydroquine	15 (37.5%)	68.87±19.54 (median, 65) (range, 46-100)	62.40±17.15 (median, 60) (range, 40-95)
Non steroidal anti-inflammatory drugs	30 (75%)	85.05±42.36 (median, 70.50) (range, 46-200)	71.33±22.42 (median, 65) (range, 32-115)

**JIA patients were receiving more than one medication*

Table 6. Comparison between serum levels of zinc and copper in relation to different treatment

	Serum zinc			Serum copper		
	Patients receiving treatment	Patients not receiving treatment	P value	Patients receiving treatment	Patients not receiving treatment	P value
Prednisone (mean±SD)	88.82±44.31	65.00±17.70	0.03*	72.09±23.31	67.03±21.40	0.57
Methotrexate (mean±SD)	71.86±16.41	93.26±57.35	0.76	68.22±21.19	73.48±24.87	0.77
Hydroquine (mean±SD)	68.87±19.54	86.50±44.98	0.25	62.40±17.15	73.13±24.49	0.75
Non steroidal anti-inflammatory drugs (mean±SD)	85.05±42.36	64.40±13.28	0.13	71.33±22.42	66.78±23.49	0.69

**P-value less than 0.05 is considered statistically significant*

were inconsistent with Çaglayan and Sukru, of zinc in active than non-active patients [20]; however, our results nearly the same as noted by Silverio et al., 2003 who reported an unchanged serum zinc level in relation to disease activity [6], these differences in results between studies could be attributed to the differences in the studied patients, different nutrition habits as serum zinc could be affected by trace elements of diet, and in the treatment given. Regarding serum copper, our results came in accordance with the same study done by Çaglayan and Sukru, 1997, in which serum copper levels were somewhat higher in active JIA compared to non-active JIA patients [20]. Moreover, Silverio et al., 2003 reported a significant higher serum copper level during disease activity [8].

In our study, the mean serum zinc and copper levels were statistically higher than controls in JIA patients in activity (p=0.022 and 0.018 respectively). However, in JIA patients in remission the mean serum zinc was 72.47±32.30 microgm/dl while in controls it was 69.96±11.40 microgm/dl, with no statistical significance between two groups (p=0.693), on the other hand, the mean serum copper was statistically higher than that of the controls (p=0.021). Comparing serum level of copper in JIA in activity and JIA in remission and control groups revealed that serum level of copper was significantly higher in JIA with activity than other two groups (p=0.036), but with no significant difference between three groups regarding serum zinc level (p=0.073). A significant high serum copper during disease activity because ceruloplasmin correlates with rheumatoid arthritis activity [6,21].

It has been reported that medications either used alone or in combination in JIA patients may have effects on serum copper and zinc levels [22]. In this study, serum levels of zinc and copper in JIA patients in relation to different medications were determined. It was observed that JIA patients on hydroquine had the lowest mean±SD serum zinc and copper levels. However, JIA patients on prednisone drugs had the highest mean±SD serum zinc and copper levels. Comparing different types of treatment in JIA patients with serum levels of zinc and copper revealed that serum levels of zinc were statistically higher in patients treated by prednisone than those not treated by prednisone (p=0.03). No statistical significance could be detected regarding other drugs. That was inconsistent with Colak etal., 2001 [23], and Onal et al., 2011 [7] who stated 1997, who noted a significant lower serum level that treatment with methotrexate elevated serum zinc levels, while treatment with salazopyrin, corticosteroids, chloquine, and nonsteroidal anti-inflammatory drugs (NSAID) did not change neither serum zinc nor copper levels. However, Milanino et al., 1993 [24] found that serum zinc level was lower in JIA patients taking NSAID and/or steroids, but drug therapy did not alter copper status in JIA patients.

5. CONCLUSION

Alteration of serum copper and zinc probably a defense response against JIA; increased copper may be due to inflammation associated, these elements could serve as biomarkers for the disease activity. Further studies are needed to correlate level of zinc and copper with the type of food taken by JIA patients.

CONSENT

Informed written Consents were obtained from every parent/surrogate of patients.

ETHICAL APPROVAL

The Scientific Research Committee of the Pediatrics Department, Cairo University approved the study protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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