Copper Differences in Egyptian Children with Developmental Stuttering

Original Article

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ABSTRACT

Background: Many previous studies claimed that people who stutter show a reduced concentration of copper in their blood. There is growing evidence about the effect of copper on basal ganglia and dystonia. Copper may also affect the dopamine and GABA systems.

Objective: This research aimed to assess free copper and ceruloplasmin levels in the serum of developmental stutterers and compare them with non- stuttering group.

Patients and Methods: The research's design was cross-sectional. It included 86 participants from the Phoniatric Unit at Minia University Hospital's Faculty of Medicine. The subjects were separated into two categories: stutterers and non-stutterers. The eighty-six participants underwent Phaonic assessment, and all were referred to a clinical laboratory for blood samples to assess ceruloplasmin and free copper. Results were statistically analyzed.

Results: The participants' mean age was 8.3±2.8 years. According to descriptive data, there is a negative association between ceruloplasmin levels and the severity of stuttering in the stuttering population. By comparing stuttering and non-stuttering groups as regards ceruloplasmin levels and estimated free copper, the mean levels of ceruloplasmin and estimated copper are significantly lower in the stuttering group.

Conclusion: This finding suggests a correlation between the degree of stuttering and the plasma levels of ceruloplasmin and copper. These results may give a new perspective on stuttering and its precipitating factors.

Key Words: Ceruloplasmin, copper(cu), stuttering.

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INTRODUCTION

Speech disorders, such as stuttering, can affect people of all ages and interfere with their ability to speak fluently in terms of time and fluency[1]. The prevalence of developmental stuttering varies by age group[7]. The most commonly cited statistic is a lifetime incidence of 5%. However, current statistics indicate a lifetime incidence closer to 10%[2,3]. Abnormalities in copper metabolism have been described in many neurological disorders, including Menkes disease and Wilson disease[8,9]. A relation between stuttering and basal ganglia dysfunction has been claimed in different research, and copper ions have been reported to alter GABA systems and dopamine levels[3,4,10]. Excess cu in the blood may result in the degeneration of dopaminergic neurons and promote oxidative stress and mitochondrial dysfunction[12]. Ninety-five percent of the total copper is bound to ceruloplasmin, and blood levels of copper and ceruloplasmin are highly correlated[12]. Pesak and Opavsky's research suggests a link between copper (Cu) and developmental stuttering. This study's outcomes revealed a decreased serum cu level in the stuttering group[5]. Stuttering-induced emotional and physical stress may raise the levels of ceruloplasmin and copper[6].

The overall goal of this study is to evaluate possible differences in measures of free copper (cu) and ceruloplasmin in children with developmental stuttering.

PATIENTS AND METHODS:

A prospective case-control study has been done between July 2022 and April 2024. One hundred participants were asked to be involved in this study after explaining the steps and aim of the study to the families. However, 86 participants agreed to do a phoniatrics evaluation and take samples. Participants were classified into two groups: stuttering and non-stuttering. Only 86 children have responded regularly to the assessment. The sample was 43 stuttering groups. Twenty-three males (age 4-12 years, mean 8.2±2.6) and 20 females (age 4-17 years, mean 8.4±3.1). The non-stuttering group was 43 children, 19 males (age 4-17, mean 8.6±3.1) and 26 females (age 4-15, mean 8.8±2.6). Consent was taken from all parents before the children were involved in this study.

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Participants

All stuttering groups are patients at the Phoniatric Unit, Faculty of Medicine, Minia University Hospital. Non-stuttering groups are children of physicians, workers, and nurses in the Phoniatric unit and Psychiatric department.

Inclusion criteria for stuttering group: The patient shows symptoms and signs of developmental stuttering

Exclusion criteria for the stuttering group were any child with inflammatory diseases, liver disease, diabetes, or medication for long periods. In addition to children with delayed language development, children with a neurogenic or psychiatric stuttering history were excluded.

Inclusion criteria for the non-stuttering group were all children with average language development ages 3-18 years.

Exclusion criteria for the non-stuttering group: children or their relatives with a stuttering and cluttering history were excluded. Any child with neurogenic disorders, language disorders, psychiatric disorders, liver disease, and diabetes was not involved in this study.

Speech assessment

speech assessment follows Ain Shams University Hospital protocol, including a complete history, auditory perceptual evaluation of spontaneous speech, and reading.

Speech samples from each child were recorded in a soundproof room, including reciting the Quran, counting 1-10, and 3 minutes of spontaneous speech. After that, the speech sample was analyzed and assessed by two Phoniatricians to evaluate stuttering and its severity. Document the severity of stuttering by using the Stuttering Severity Instrument[13]. The SSI Arabic version evaluates the degree of stuttering in adults and children. SSI Arabic version is reliable, valid, and standardized for children and adults. The stuttering severity instrument includes the following parameters:-

A. The frequency of stuttering events is transformed into a scale score (ranging from 4 to 18).

B. Estimated duration of the longest stuttering occurrence (converted to a score of 0-18).

C. physical concomitants that can be observed and graded on a scale of 0 to 20. The three parameters' scores-frequency, duration, and physical concomitants-are summed to obtain an overall severity score. The severity of stuttering can be determined by comparing this score to the age-appropriate normative values in conversion tables. A percentile or grade (very mild, light, moderate, severe, or severe) might represent the score.

All participants were referred from the Phoniatric Unit to the Clinical Laboratory at Minia University Hospital for a blood sample.

Biomedical method

Venous blood samples were withdrawn under complete aseptic conditions. After clotting for 20 minutes at room temperature, the sample was centrifuged for 20 minutes at 300 RPM. Serum copper level was assayed via colorimetric method at 440nm by spectrophotometer. The leftover serum was kept at -80 °C, and the ceruloplasmin level was determined using an enzyme-linked immunosorbent assay (ELISA) with absorbance at 450 nm and monospecific antibodies against human ceruloplasmin (CP). Estimated free copper was calculated using equation = total copper in serum (μg/dl) – 3.15x ceruloplasmin (mg/dl)

Statistical method

Data entry and statistical analysis were performed using the SPSS statistical software package for Windows, version 20.0 (IBM *et al*., USA). Our analytical results provide the data using descriptive statistics, such as frequencies and percentages for qualitative variables and mean and standard deviations for quantitative variables. Chi-square tests and ANOVA were employed to assess the statistical significance of correlations between categorical data. An independent t-test was used. A nonparametric test (Mann Whitney) was used to determine the age of the first speech word and sentence.

RESULTS:

Descriptive data

Demographic characteristics of the studied group

This research was done on a sample of 100 Arabic-speaking Egyptian children. Fourteen participants still need to complete the clinical laboratory test. Thus, the final participants were 86 children, 44 females (51.2%) and 42 males (48.8%) between 4 and 17 years old with a mean age of 8.5±2.8. Those participants are classified into two groups. Stuttering group of 43 children (23 males, 20 females) and 43 non-stuttering (19 males, 26 females) (Table 1).

Stuttering Group

The age of starting stuttering in the group ranges from 2 to 10 years. After evaluating the stuttering degree using SSI (Arabic version), the stuttering group is divided into five subcategories ranging from very mild to profound (Table 2). The average length of the stuttering event is 6 seconds (see Table 3)

An independent t-test was performed to compare free copper and ceruloplasmin levels and estimated free copper in the stuttering and non-stuttering groups.

A substantial difference was seen in the mean of free copper, ceruloplasmin level, and estimated free copper between the stuttering group (total copper mean (in micromole per liter) = [11.27], SD = [1.17], *P* = [0.001], estimated free copper mean (in micromole per liter) = [4.3], SD = [1.01], *P* = [0.001], Ceruloplasmin level means (in gram per liter) = [0.26], SD = [0.05], *P*= [0.001]) and non-stuttering group (total free copper mean (in micromole per liter) = [18.14], SD = [2.31], *P* = [0.001], estimated free copper mean (in micromole per liter) = [5.1], SD = [0.75], *P* = [0.001], Ceruloplasmin level mean (in gram per liter) = [0.29], SD = [0.06], *P*= [0.001]) (Table 4).

Note: p-cu (plasma copper) average value in children (11:25), p-cp (plasma ceruloplasmin) average value in children (0.24-0.46)

A one-way ANOVA was used to compare the effects of stuttering severity on ceruloplasmin levels. A statistically significant difference in the mean level of ceruloplasmin between the five groups that represent stuttering severity (F (25.4) df (4), *p*= (.001) (Table 5)

Note. mean ceruloplasmin level is calculated in grams per liter

A two-sample t-test was performed to compare ceruloplasmin levels in the male and female groups in the studied sample. Ceruloplasmin levels did not differ significantly between males (0.28±0.05) and females (0.29±0.05) (df (84) = [.551], *p*= [0.63]).

Table 1: Characteristics of Study Group

	Stuttering 43(50)	Non-stuttering 43(50)	*p-value*
Gender			
Males (%)	23 (53.5%)	19 (44.2)	0.20
Females (%)	20 (46.5%)	26(55.8)	
Age (mean± SD.)	8.3± 2.8	8.7± 2.8	0.49
1st word age (mean± SD.)	1.04 ± 0.16	1.10 ± 0.24	0.11
1ST Sentence age (± SD.)	1.99 ± 0.2	1.96 ± 0.09	0.30

Table 2: Classification of Stuttering Group According to Stuttering Severity Index Arabic version (SSI)

Severity	Stuttering Group N (43)	
	frequency	Percentage
Very mild	10	23.3
Mild	12	27.9
Moderate	9	20.9
Severe	7	16.3
Profound	5	11.6

Table 3: Characteristics of Stuttering Group

	Range	Mean ± SD.
Start age of stuttering (years)	2-10	5.4 ± 1.9
Length of a stuttering event (seconds	2-10	6.1 ± 4
Duration between the start of stuttering and seeking medical advice (months)	1-5	2.8 ± 1.1

Table 4: Free copper, Ceruloplasmin level, and estimated free copper in the stuttering and non-stuttering groups.

Group	(P-cu, µmol/l)	(p-Cp, g/l)	(Estimated free Cu, µmol/l)
Stuttering	11.27±1.17	0.26±0.05	4.3±1.01
Non-stuttering	18.14±2.31	0.31±0.04	5.78±0.75
total	15.1±3.9	0.29±0.06	5.1±1.12
p-value	0.001	0.001	0.001

Table 5: Ceruloplasmin level and severity of stuttering.

Stuttering Severity	Ceruloplasmin level N (43)			
	Mean±SD	Range	CI	*p-value*
Very mild	0.30±0.03	0.27:0.36	0.28:0.32	0.001
Mild	0.31±0.03	O.29:0.33	0.29:0.33	
Moderate	0.26±0.04	0.20:0.36	0.23:0.30	
Severe	0.21±0.007	0.17:0.20	0.20:0.21	
Profound	0.18±0.01	0.17:0.20	0.17:0.20	

DISCUSSION

The findings of this study demonstrated a link between developmental stuttering in children and low plasma levels of cu and ceruloplasmin. A previous study by Pesak and Opavsky on sixteen male stutterers with developmental stuttering found a statistically significant reduction in serum copper and a negative link between stuttering severity and copper. Similarly, in our study, serum copper and ceruloplasmin levels were lower in the stuttering group than in the control group. There was a negative association between ceruloplasmin level and stuttering severity, consistent with Pesak and Opavsky's study findings. Stress affects serum copper and ceruloplasmin levels, which explains this.

Our study demonstrated reduced plasma Cu and serum Ceruloplasmin, which increased inactive copper (estimated free copper). Disturbance in copper metabolism increases inactive form concentration in tissues and neurological tissues. Abnormal copper metabolism is hypothesized to yield metabolic disturbances in basal ganglia involved in focal dystonia. According to this finding, developmental stuttering may be a type of focal dystonia, and this is agreed with Takahiro Mezaki *et al*. (2001)[16], who reported that abnormal copper metabolism is a factor involved in developing primary.

Dystonia. Kiziltan and Akalin (1996)[17] reported that his study provides additional evidence to support the concept that stuttering is a focal or segmental action dystonia. Additionally, agreed with Fletcher *et al*. (1991)[18], who reported that Idiopathic torsion dystonia individuals are more likely to have a family history of stuttering.

Against our results, previous research by Per A. Alm[11] that studied Cu and Ceruloplasmin levels in adult stutterers proved a preference for a positive correlation between copper and ceruloplasmin and the severity of stuttering. This finding may be due to heterogeneity in methodology and the study of different age groups.

Additionally, plasma Cu levels were generally lower when compared to prior investigations of healthy children. In this investigation, the mean copper for stuttering was 11.27 µmol/l, compared to the usual copper 18.6[14]. These findings could be explained by heterogeneity within the stuttering group. Stress may affect levels of Cu and ceruloplasmin, and cu may be increased by physical and traumatic stress[15]. It is theoretically possible that stuttering stress could increase the ceruloplasmin and Cu levels. However, in our study, the Cp and Cu levels are low compared to the control and the normal range. It can be explained by the mechanism of Cu's response to stress in children being different from that in adults.

CONCLUSION

This study explores the relationship between stuttering severity, serum Cu, and serum ceruloplasmin. The current study's findings suggest a link between developmental stuttering in children and low plasma Cu levels. These findings are intriguing because stuttering is a basal ganglia disorder with dystonia-like characteristics.

ABBREVIATIONS

Copper (Cu)

Ceruloplasmin (Cp)

Stuttering Severity Instrument (SSI).

CONFLICT OF INTEREST

There are no conflicts of interest.

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