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# The concentrations of bioelements in the hair samples of Jordanian children who stutter

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ABSTRACT

*Objectives:* This study investigates the levels of 15 bioelements (calcium, copper, chromium, sodium, iron, magnesium, manganese, zinc, cobalt, selenium, molybdenum, vanadium, potassium, boron, and lithium) in the hair species of Jordanian stutterer.
*Methods:* The subjects of the study included 25 cases of stuttering, and 25 normal children (age and sex matched). The severity of stuttering (low, moderate, and severe) were assessed using Stuttering Severity Instrument, Fourth edition (SSI-4). Hair samples of subjects were cut, washed, dried, physically degraded, hydrolyzed, and analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).
*Results:* Results indicated that the levels of bioelements (Calcium, Copper, Chromium, Magnesium, Manganese, Cobalt, Selenium, Molybdenum, Vanadium, Boron, and Lithium) were significantly lesser in the hair samples of stuttering group than the control group.
*Conclusion:* The findings of the current study could support the use of biochemical analyses as diagnostic biomarker for stuttering.

## 1. Literature review

Stuttering is a high frequent disturbance in the smoothness of speech that is described by (a) repetitions of words, syllables, and sounds, (b) elongations of sounds, and (c) obstruction in the flow of speech [1,2]. These behaviors usually associated with physical tension and high speech rate [2]. The onset of stuttering could arise at any time, but mostly between the age of 2 and 5 years [2–5]. Stuttering is a complex disorder and the exact cause of this phenomenon is unknown; some approaches describe stuttering physiologically or biologically [4,6–9], psychologically [10], genetically [11–16], neurologically and motor control [17–21] and learned behavior [4,22,23]. A number of treatments programs are available to decrease stuttering, most of these programs are behavioral. They are designed to teach the person who stutters certain skills or behaviors to improve fluency and reduce the rate of speech to monitor breathing. Also, the goal of therapy is to eliminate physical tension, psychological behaviors, and social anxiety or avoidance associated with stuttering [24–28]. Bioelements play an essential function in human health and disease. They are structural components of biological molecules such as enzymes, vitamins, and proteins. They participate in different reactions at tissue, cellular, and sub-cellular levels. These include immune-regulation, nerve conduction, muscle contraction, membrane potential regulation, mitochondrial activity, protein and nucleic acid metabolism, activation of enzyme functions [29–32]. Therefore, imbalances in the levels of bioelements may negatively affect biological pathways, and are associated with many disorders [33]. It is of interest, then, to study the association between stuttering and the concentrations of bioelements in an attempt to address the cause of stuttering and either decrease or increase the levels of these elements to improve fluency.

There have been many attempts to use hair concentrations and blood samples to assess different types of disorders and exposures. For example [34], conducted a review article reporting hair concentrations of children exposed to a drug, they found that the concentrations of cocaine, codeine, 6-AM and morphine are higher in hair from in utero exposure compared to children exposed passively, however, methamphetamine showed no significant difference between passive and utero exposure. Reference [35] evaluated the GPER-1 level in the blood samples of 30 patients with developmental stuttering (2 males, 6 females). The results of the study showed that the GPER-1 levels of the patients with a stutter are statistically significantly higher than those of the control group with high sensitivity and specificity indicating that GPER-1 levels could be an important factor in the diagnosis and treatment of stuttering.

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Due to the significant impact of bioelements on neurotransmitters and antioxidant defense system, the levels of such elements were linked to neurological, psychological, and behavioral disorders. For example, it was found that the hair of mentally retarded group had significantly high levels of lead and cadmium [36], higher levels of calcium [37], and lower levels of iron, copper, and magnesium [37].

The hair of patients with epilepsy were also studied in reference to normal subjects. Results showed that copper, magnesium, and zinc were significantly lesser in epileptic cases than the hair of healthy subjects [38]. The concentrations of bioelements in hair species of schizophrenic cases were assessed in the previous literature. Results indicated that the concentration of Zinc and Calcium reduced significantly whereas the concentration of copper and Cadmium were significantly higher in schizophrenic subjects while the concentration of Manganese remain unchanged [39].

Reference [40] assessed the levels of zinc, silicon, magnesium, iron, copper and calcium in the hair samples of 81 subjects with Parkinson's disease. Results revealed significantly lesser concentrations of iron in the hair of cases with Parkinson's disease compared with normal subjects. Results also showed that the concentrations of Calcium and Magnesium were lower while the concentration of Zinc was higher in the hair of patients.

Although considerable research has been conducted to examine the levels of bioelements in the samples of patients with neurological and psychological disorders, there is still a dearth of data about the levels of these elements in the hair of cases with communication disorders such as stuttering. One of these studies compared sixteen men with developmental stuttering with sixteen men without speech impairments to investigate the relation between copper and the severity of stuttering, no relation was found between copper and stuttering [41]. On the other hand, a possible relation was found between copper and developmental stuttering in the study of [42] who found a significantly lower level of serum copper in person with stuttering than controls. Calcium and phosphorus were reported to be high in stutterers [43], whereas normal amounts of calcium and potassium were indicted in stutterers [44].

It is clear that only few single elements were examined with relation to stuttering and controversial results were found. To my knowledge, this is the first study that examines the levels of 15 bioelements (sodium, chromium, magnesium, copper, iron, manganese, calcium, zinc, cobalt, selenium, phosphorus, and molybdenum) in the hair species of Jordanian people who stutter. This study aims at examining the levels of 15 bioelements (calcium, copper, chromium, sodium, iron, magnesium, manganese, zinc, cobalt, selenium, molybdenum, vanadium, potassium, boron, and lithium) in the hair species of Jordanian people who stutter.

## 2. Methodology

### 2.1. Study design and data collection

The presented research study was approved by King Abdullah University Hospital institution review board (IRB no. 10/215/2444). The 25 participating children who stutter between age of 3 years and 8 years who attend the speech clinic at King Abdullah hospital in Jordan were selected for the purpose of the current study. The control group of 25 normal fluent children were selected from the same families of the stuttering group. Certified speech pathologist screened cases and controls for speech and language impairments as well as hearing evaluation to exclude those who have other communication disorders such as articulation, neurological dysfunction, or hearing loss. Speech samples were collected from case group to diagnose the severity of stuttering using Stuttering Severity Instrument (SSI-4) by certified speech pathologist (Table 1). The subjects of both case and control groups were average age-matched, sex-matched, and region of origin-matched. Family based controls were selected of similar age and gender for case group. Children typically have very similar diet and environmental exposure to other families' members therefore, comparing children who stutter to their siblings could enable us to correlate levels of bio-elements with stuttering.

**Table 1**
Participants information.

| Participant | Age | Gender | Diagnosis | Treatment | SSI Scores | Severity Rating |
|---|---|---|---|---|---|---|
| **1** | 7 | M | Stuttering | No treatment | 7 | Very mild |
| **2** | 7 | M | Stuttering | No treatment | 9 | Very mild |
| **3** | 7 | M | Stuttering | No treatment | 7 | Very mild |
| **4** | 4 | F | Stuttering | No treatment | 9 | Very mild |
| **5** | 4 | M | Stuttering | No treatment | 10 | Very mild |
| **6** | 5 | F | Stuttering | No treatment | 7 | Very mild |
| **7** | 8 | F | Stuttering | No treatment | 7 | Very mild |
| **8** | 7 | M | Stuttering | No treatment | 14 | Mild |
| **9** | 3 | M | Stuttering | No treatment | 14 | Mild |
| **10** | 3 | M | Stuttering | No treatment | 12 | Mild |
| **11** | 4 | M | Stuttering | No treatment | 17 | Mild |
| **12** | 8 | F | Stuttering | No treatment | 14 | Mild |
| **13** | 8 | M | Stuttering | No treatment | 22 | Moderate |
| **14** | 7 | M | Stuttering | No treatment | 25 | Moderate |
| **15** | 7 | M | Stuttering | No treatment | 26 | Moderate |
| **16** | 8 | M | Stuttering | No treatment | 22 | Moderate |
| **17** | 5 | F | Stuttering | No treatment | 25 | Moderate |
| **18** | 4 | M | Stuttering | No treatment | 21 | Moderate |
| **19** | 4 | M | Stuttering | No treatment | 22 | Moderate |
| **20** | 7 | M | Stuttering | No treatment | 33 | Severe |
| **21** | 5 | M | Stuttering | No treatment | 29 | Severe |
| **22** | 5 | M | Stuttering | No treatment | 29 | Severe |
| **23** | 3 | M | Stuttering | No treatment | 33 | Severe |
| **24** | 3 | F | Stuttering | No treatment | 33 | Severe |
| **25** | 5 | F | Stuttering | No treatment | 29 | Severe |

In the clinic, the parents were signed the assent forms to permit their children to participate in the project and answered interview questionnaires about age, sex, biometric information, health status, hair care, cosmetic treatment, life style, geochemical environment, region of origin, drinking water, and dietary habits, etc. A sample of 1 cm length of scalp hair were cut from the occipital area of each subject to maintain the physical appearance. Each hair sample was placed in a sealed plastic baggie labeled with subject's identification number. Hair samples used in this study were specified no dye treatments, bleaching, or any other chemical treatment besides conditioners. This study is blinded in which the researchers did not know the identity of the samples in terms of whether they come from subjects who stutter or not.

### 2.2. Sampling and pre-analysis preparation

Three sub-samples of hair for each individual were prepared for analysis. The determination of bio-elements is a multidimensional analytical method, including cleaning, pulverization, digestion, and ICP-MS analysis.

#### 2.2.1. Washing and pulverization

Preceding analysis, hair samples were cleaned to remove surface lipid, dust, shampoo residues, sweat, and exogenous contamination containing elements of interest. The hair sample was transferred into sterile 50 ml polypropylene tube, rinsed with 0.1% Triton X 100 solution and vortexed for 10 min (Barnstead International, Dubuque, IA, USA). After decanting Triton X, the sample was rinsed three times with DI water. The sample was then washed three times with acetone; vortexed for 10 min. Acetone was poured off and washed hair was dried at 70 °C overnight in a drying cabinet to get rid of excess solvents (LTE Scientific. Greenfield, Oldham, UK). The dried samples were frozen in liquid nitrogen at −196 °C for 45 min then were pulverized to make powder using a mini bead beater-16, model 607EUR (BioSpec Products, Bartlesville, OK, USA). The fine powder was then separated into three sub-samples of 300 mg.