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Dopamine and Serotonin Levels in Blood Samples of Jordanian Children Who Stutter

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***Abstract*—**This study examines the levels of dopamine and serotonin in blood samples of children who stutter compared with normal fluent speakers. Blood specimens from 50 children who stutter (6 females, 44 males) and 50 normal children matched age and gender were collected for the purpose of the current study. The concentrations of dopamine and serotonin were measured using the 1100 series high-performance liquid chromatography coupled with ultraviolet detector instrument (HPLC-UV). It was revealed that dopamine level in the blood samples of stuttering group and fluent group was not significant (P = 0.769), whereas the level of serotonin was significantly higher in the blood samples of stuttering group than the blood samples of fluent normal group (P = 0.015). It is concluded that serotonin blockers could be used in future studies to evaluate its role as a medication for the treatment of stuttering.

***Keywords*—**Dopamine, serotonin, stuttering, fluent.

I. Introduction

NEUROTRANSMITTERS, which link neurons with each other, have important roles in normal development of memory, motor activity and behavior regulation [1]. According to these important functions, dysfunction in the neurotransmitter system is thought to be the cause of many developmental disorders, by affecting the migration of neuronal cell, synaptogenesis and differentiation and ultimately developmental processes of the brain [2].

Elevated levels of neurotransmitter dopamine have been found among adult people who stutter, and have consequently found dopamine antagonists that reduce stuttering [3]. Using functional and diffusion imaging, overactivity has been found at the level of the substantia nigra and extended to the pedunculopontine nucleus, red nucleus and subthalamic nucleus, which all contribute to the production of dopamine [4].

Costa & Kroll provided recommendations for physicians who need to apply medical findings to treat people with developmental stuttering. They stated that the severity of stuttering has been decreased using a serotonin-dopamine antagonist compared with placebo, they concluded that the theory of a hyperdopaminergic causation of stuttering could be supported by the effectiveness of dopamine receptor antagonists in developmental stuttering [5].

In an earlier study, dopamine levels were examined in the striatum of three male people who stutter (moderate to severe) compared with levels in six normal fluent speakers [6]. Results indicated that dopamine levels in stuttering subjects showed a 100-300% increase in ventral limbic cortical and sub cortical areas which are related to the hypotheses that stuttering is associated with an overactive pre-synaptic dopamine system in these regions of the brain [6].

Maguire et al. evaluated the effectiveness of risperidone in the treatment of developmental stuttering. Risperidone works in the brain and affects various neurotransmitters, in particular dopamine and serotonin, it works by blocking the receptors in the brain that dopamine acts, which in turn prevents the extreme activity of dopamine. It was indicated that measures of stuttering severity decreased significantly in the risperidone group than in the placebo group ($p < 0.05$). It is concluded from this study that risperidone may be effective in the treatment of developmental stuttering [7].

In another study, the efficacy of olanzapine (dopamine blocker) in the treatment of developmental stuttering was examined compared with placebo [8]. Stuttering severity Instrument (SSI-3) and a subject-rated self-assessment of stuttering (SSS) were used to evaluate the subjects. Results indicated that olanzapine was statistically effective in the treatment of stuttering on the three ratings of stuttering severity compared with placebo. It is concluded from this study that olanzapine as a blocker for dopamine activation could be a promising medication for the treatment of stuttering [8].

Another dopamine antagonist that was used in the treatment of stuttering was Haloperidol. It is a precursor to the serotonin-dopamine antagonists that blocks dopamine-2 receptor in the brain. As a result, haloperidol stops the effects of dopamine in the synaptic cleft and reduces dopaminergic transmission between neurons. The efficacy of this medication was evaluated by [9] and it was found that haloperidol was more effective than placebo in improving fluency. The relationship between serotonin and stuttering has been discussed in very few studies. For example, the use of serotonin reuptake inhibitor (paroxetine) has been evaluated in the management of stuttering symptoms and found to be effective [10]. Consequently, it is speculated that one of the main neural systems associated with developmental stuttering may rely on the potential dysfunction of the basal ganglia system.

These studies provide some support for the hypothesis that dopamine level could be involved in stuttering and if over-activation of dopamine was found, dopamine blockers could be the future promising medications for developmental

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stuttering. We hypothesize in the current experiment that elevated levels of both dopamine and serotonin may explain the neural background of developmental stuttering Thus, the purpose of this study is to examine the level of dopamine and serotonin in the blood samples of people who stutter compared with normal fluent speakers. The findings of such study could be the base for further biochemical studies that shed some light on the linkage between dopamine and serotonin neurotransmitters in blood samples of people who stutter, if such relation was confirmed, further studies could be conducted to evaluate the role of neurotransmitter blockers in the treatment of stuttering.

II. Materials and Methods

A. Participants

An approval to conduct the study was obtained from the institution review board (IRB) in King Abdulla University Hospital (20/108/2017). The consent forms were signed by one of the parents who agreed to participate in the study prior filling out questionnaires regarding their children's demographic information and their language abilities.

The children who stutter (CWS) are 50 subjects (6 females, 44 males). They were randomly selected from the speech clinic at KAUH of aged between 4 and 15 years (M = 7.9; SD = 3.3). Those who have any kind of neuropsychiatric disorders such as depression, attention-deficit/hyperactivity disorder (ADHD), Tic disorders, autism spectrum disorder, speech/ language disorders, learning disorders, schizophrenia, bipolar and related disorders, depressive disorders, and anxiety disorders were excluded from the study. The control group included 50 healthy fluent children (age and sex matched).

B. Diagnosis and Measurement of the Severity of Stuttering

The principal investigator who is a speech-language pathologist diagnosed CWS and rated their severity using Stuttering Severity Instrument–Fourth Edition (SSI-4) [11]. The stuttering severity is classified into very mild, mild, moderate, and severe based on the scoring at four areas of speech status: frequency, duration, physical concomitants, and naturalness of the individual's speech [11].

C. Blood Sampling and Extraction of Dopamine and Serotonin

Blood specimens (3 mL) from the participants were collected in speech clinic at KAUH. After 15 min of centrifugation (4500 rpm, 4 °C), the plasma layer was transferred into polyethylene tube and stored it at –70 °C until analysis. The samples were analyzed within 3 weeks after the collection. 2 mL of mixture of acetonitrile and methanol (1:1) was added to 1 mL of plasma. The mixture was vortexed for 2 min, then centrifuged at 4500 rpm for 10 min at 4 °C. 1 mL of supernatant was transferred into HPLC vial [12], [13]. This process of blood sampling in addition to selecting the participants and measure their severity was conducted from October, 2017 to January, 2018.

D. Instrumentation

The 1100 series HPLC-UV (Agilent Company, USA) was used for measuring the plasma concentrations of dopamine and serotonin. Three replicates of plasma samples for each subject were prepared and injected into HPLC on three separate days to increase the precision of the measurement. Measuring dopamine and serotonin took about three weeks (in February, 2018).

E. Data Analysis

The analyses of the data included measuring peak areas of dopamine and serotonin versus standard concentrations. Plasma concentrations for each of dopamine and serotonin in subject samples were calculated from the respective calibration standard curves. The concentrations of dopamine and serotonin (mg/mL) in the two groups (stutter and healthy) were compared using one-way analysis of variance (ANOVA). The receiver operating characteristic curve (ROC) was also generated using IBM SPSS software version 21.

III. Results

One-way ANOVA was run to compare mean levels of dopamine and serotonin in the blood samples of both fluent group and stuttering group, these results are presented in Table I.

TABLE I
Mean and Standard Deviations of Dopamine and Serotonin Levels in Both Stuttering Group and Fluent Group

	Stuttering group M±SD	Fluent group M±SD	Effect size	*P*-value
Dopamine	0.15 ± 0.01	0.01 ± 0.01	0.000	0.769
Serotonin	0.20 ± 0.05	0.18 ± 0.06	0.153	0.015

Results indicated that the level of dopamine in the blood samples of both stuttering group and fluent group was not different (P = 0.769). On the other hand, it was shown that the level of serotonin in the blood samples of stuttering group was significantly higher than the blood samples of fluent normal group (P = 0.015). Full details about the patients group (age, gender, severity of stuttering, dopamine level, and serotonin level) are presented in Table II.

ROC was used to evaluate the diagnostic performance of serotonin levels (12). In this curve, sensitivity as true positive rate was plotted in function of the false positive rate (100-Specificity) (Fig. 1).

The area under the ROC curve is a measure of how well the level of serotonin can distinguish between two diagnostic groups (stuttering/fluent). The area in the current model is equal to 0.6 indicating that serotonin level could be a good variable distinguishing between the two groups. It is also possible that this area could be close to 1 by increasing the sample size of the study indicating a perfect separation of the values of the two groups.

Level of serotonin was compared with the fourth categories of stuttering severity (very mild, mild, moderate, and severe) using one-way ANOVA. Results indicated that the mild patients were significantly different from the other ratings of

severity ($P \leq 0.001$). These results are presented in line graph (Fig. 2) which shows that the level of serotonin increases with higher ratings of severity.

TABLE II
DETAILS ABOUT PATIENT GROUP (AGE, GENDER, SEVERITY, DOPAMINE LEVEL, AND SEROTONIN LEVEL)

Participant	Age	Gender	SSI Scores*	Severity Rating	Dopamine Level (mg/mL)	Serotonin Level (mg/mL)
1	4	M	12	Very mild	0.03	**0.19**
2	6	M	9	Very mild	0.07	**0.21**
3	8	M	11	Very mild	0.03	**0.18**
4	10	M	31	Severe	0.04	**0.07**
5	13	M	21	Moderate	0.03	**0.18**
6	5	F	18	Mild	0.05	**0.22**
7	15	M	21	Moderate	0.03	**0.20**
8	9	M	16	Mild	0.03	**0.21**
9	7	M	17	Mild	0.03	**0.21**
10	5	F	23	Moderate	0.04	**0.18**
11	11	M	29	Severe	0.04	**0.07**
12	8	M	15	Mild	0.04	**0.13**
13	8	M	25	Moderate	0.03	**0.05**
14	4	M	24	Moderate	0.02	**0.04**
15	5	M	17	Mild	0.04	**0.13**
16	8	M	20	Moderate	0.03	**0.08**
17	12	M	33	Severe	0.04	**0.07**
18	6	F	7	Very mild	0.04	**0.19**
19	11	M	12	Mild	0.05	**0.22**
20	12	M	22	Moderate	0.04	**0.20**
21	9	M	21	Moderate	0.03	**0.19**
22	7	F	26	Moderate	0.03	**0.20**
23	10	F	9	Very mild	0.03	**0.19**
24	5	F	6	Very mild	0.03	**0.21**
25	4	M	25	Moderate	0.02	**0.10**
26	11	M	27	Moderate	0.02	**0.10**
27	12	F	19	Mild	0.02	**0.20**
28	6	M	15	Mild	0.01	**0.20**
29	4	M	30	Severe	0.01	**0.20**
30	6	M	34	Severe	0.01	**0.19**
31	9	M	24	Moderate	0.01	**0.16**
32	13	M	31	Severe	0.01	**0.16**
33	10	M	11	Mild	0.009	**0.34**
34	9	M	7	Mild	0.01	**0.078**
35	13	M	17	Mild	0.01	**0.16**
36	7	M	23	Moderate	0.09	**0.19**
37	7	M	24	Moderate	0.06	**0.24**
40	11	M	34	Severe	0.07	**0.22**
41	9	M	13	Mild	0.01	**0.17**
42	6	M	28	Severe	0.01	**0.29**
43	4	M	9	Very mild	0.01	**0.19**
44	8	M	21	Moderate	0.01	**0.17**
45	8	M	31	Severe	0.007	**0.12**
46	4	M	18	Mild	0.06	**0.17**
47	4	M	24	Moderate	0.03	**0.19**
48	9	M	31	Severe	0.05	**0.21**
49	5	M	29	Severe	0.03	**0.18**
50	4	M	7	Very mild	0.04	**0.16**

*The frequency of stuttering + the average of three longest moments of stuttering + the number of physical concomitants

IV. DISCUSSION

The purpose of this study is to measure the level of dopamine and serotonin in the blood samples of people who stutter compared with normal fluent speakers. The findings indicated that dopamine level was higher in stuttering group compared to normal group, but it was not significant. Regarding serotonin level, it was found significantly higher in stuttering group than fluent normal group. These findings are not compatible with previous studies who found that higher

levels of neurotransmitter dopamine have been found among people who stutter, and have accordingly found dopamine antagonists that improve fluency [3], [5]-[9].

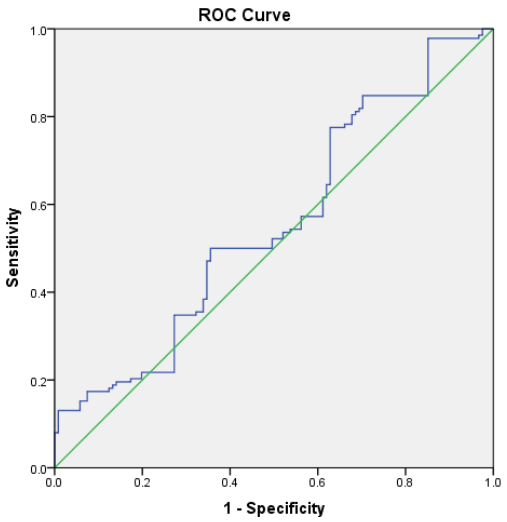

Fig. 1 ROC curve of serotonin level

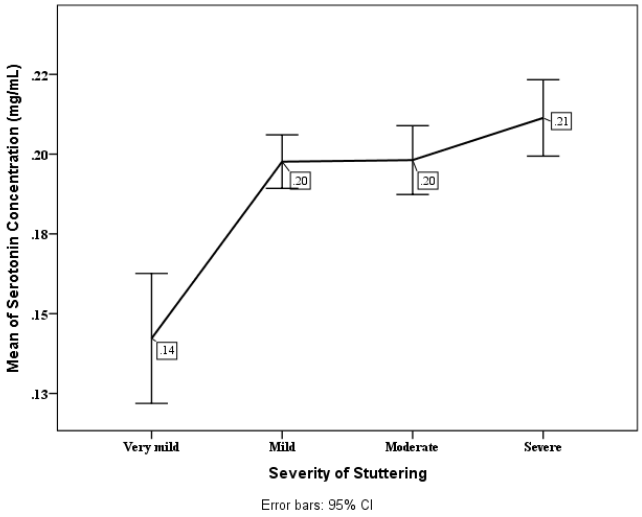

Fig. 2 Dopamine and serotonin levels compared with severity of stuttering

Stuttering is a multifaceted speech disorder that includes core behaviors of frequent prolongations, repetitions of word/ syllable/sound, silent blockages [15]-[17], as well as secondary behaviors that are learned in response to the core behaviors such as eye blinks, head movements, finger snaps [15], [18], [19]. Lower level of serotonin was found in the blood samples of patients who were rated as very mild in the SSI-4, patients with this category of severity show less frequent symptoms of disfluencies in both core behaviors (prolongations, repetitions of word/syllable/sound, silent blockages) and secondary behaviors (eye blinks, head movements, finger snaps). On the other hand, patients with severe stuttering who registered higher scores of disfluencies showed higher level of serotonin.

A link between the abnormality of basal ganglia and stuttering has been suggested [20], [21]. The basal ganglia are important structures of subcortical gray matter situated in the forebrain and affect emotion, cognition, and motor behavior [20]. It was proposed that the basal ganglia fail to produce precise and correct timing cues for initiation of the next motor segment in speech [21]. Reference [4] has also indicated an overactivity in the midbrain at the level of the substantia nigra, the area where dopamine neurons are located. The function of basal ganglia is strongly controlled by dopaminergic activity [24].

Neurotransmitters are brain chemicals that transfer messages throughout the brain by sending it between neurons through synapses [25]. So far within the stuttering domain the main focus has been on the neurotransmitter dopamine and serotonin. The findings of the current study have been supported previously by [26] who suggested elevated dopamine levels in the brains of people who stutter.

Improving fluency through the use of certain drugs that block certain dopamine and serotonin receptors in the brain support the dopamine hypothesis in stuttering [27], it was proposed that disfluency could result from hyperactivation in the dopaminergic system [6], [28]. However, no significant differences were reported in dysfluency of Parkinson disease in low and high dopamine level [29].

The findings of the current study in field of neurotransmittors and stuttering suggests that changes in the concentration of neurotransmitters (dopamine and serotonin) in the human brain may cause a variety of pathological states such as disfluency [3], [6], [7]. Such studies on the dopamine-serotonin hypothesis in stuttering suggest that a well-regulated dopamine release is vital for the appropriate function of the basal ganglia. Higher release of dopamine or serotonin would lead to general inhibition of movements and impulses that might cause a dysfluency of speech.

The real cause of dysfluency in people who stutter is not fully understood yet. However, examining the concentration levels of dopamine and serotonin in blood samples of people who stutter may explain the underlying mechanisms of stuttering to a certain extent and provide biochemical information on the region of interest, which may open the door for further studies that evaluate dopamine and serotonin blockers in the treatment of stuttering. Specifically, a scientific advantage of the current study is trying to clarify the neuronal basis of stuttering by looking at the brain neurochemistry using dopamine and serotonin levels. In particular, we looked at the neurotransmitters as significant possible candidates for understanding the biochemical manifestations of stuttering. Nevertheless, our findings need further research with larger sample sizes and control groups investigating the levels of other brain neurotransmitters in addition to dopamine and serotonin.

Dopamine is an organic chemical that is synthesized in both kidneys and brain. In brain, it functions as a neurotransmitter and it is affected by many factors such as motivational behavior, addictive drugs [30], in addition to the diseases of the nervous system that are usually associated with dysfunctions of the dopamine system [31]. L-DOPA, which is

the direct precursor of dopamine that can be manufactured from either an essential amino acid (phenylalanine) or from a non-essential amino acid (tyrosine), these amino acids are found in almost every protein and so are obtainable from food [32]. Deficiency in any required amino acid or cofactor can damage the synthesis of dopamine [32]. Age is also an important factor related to dopamine and serotonin levels; a number of studies found a reduction in dopamine with aging [33]. Serotonin is synthesized from the amino acid L-tryptophan [34]; therefore consuming foods with purified tryptophan affects the level of serotonin [35]. Various physiological and psychological process affect and are moderated by the serotonin level such as the feelings of happiness, reward, cognition, memory, learning [36]. Serotonin is also known to be associated with aging, learning and memory. The level of serotonin increases during early stages of life which changes the behaviors of moving independently [37].

V. Conclusion

The current research observes the concentrations of dopamine and serotonin in blood samples of CWS compared with normal speakers using the 1100 series HPLC-UV. It was found that level of serotonin was significantly higher in stuttering group than the normal group, whereas the level of dopamine was not significant between them. Consequently, it is concluded that the role of serotonin as a medication for the treatment of stuttering could be evaluated in further studies through the implication of serotonin blockers.

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