Assessment of Vitamin D Level in Preschool Children who Stutter

Hazem Alawadli[1], Omar El Henawy[2], Hanan A. Mohamed[3], Abdallah M. AbdElzaher[3], Shaimaa Mostafa[4]

Original Article

[1]Phoniatric Unit, ORL Department, [2]Clinical Pathology Department, Faculty of Medicine, Aswan University, Egypt.

[3]Phoniatric Unit, ORL Department, Faculty of Medicine, Assuit University, Egypt.

[4]Phoniatric Department, Hearing and Speech Institute, General Organization for Teaching Hospitals and Institutes, Egypt.

ABSTRACT

Background: Stuttering, affecting approximately 5% of children, disrupts speech flow and poses challenges to psychosocial development. Its complex origins involve genetic, neurological, environmental, and psychological factors. This study aims to explore the potential link between stuttering in preschoolers and Vitamin D(VD) deficiency, a relatively unexplored area.
Objectives: To study the association between stuttering in preschool-aged children and insufficient VD levels (specifically, 25-hydroxy cholecalciferol) in order to assign vitamin D deficiency as contributing factor for stuttering occurrence.
Patients and Methods: A case-control study was conducted on 36 preschool Arabic-speaking children aged 4-6 years, employing a 2:1 design with 24 stutterers and 12 age- and sex-matched healthy controls. Stuttering severity was assessed using The Arabic Stuttering Severity Index, and serum vitamin D levels were evaluated using 25-hydroxy cholecalciferol.
Results: Results indicated a higher prevalence of vitamin D deficiency in children who stutter compared to controls, though no statistically significant difference was found in the distribution of vitamin D classifications between the two groups. Additionally, there was a non-significant association between Arabic Stuttering Severity Index categories and vitamin D categories among children who stutter. Severe stuttering cases exhibited higher rates of vitamin D deficiency compared to insufficient levels, and a statistically significant negative correlation was observed between vitamin D levels and Arabic Stuttering Severity Index score valuess.
Conclusion: Stuttering is a common health concern, particularly among preschool-aged children. Lower vitamin D levels were observed in children who stuttered compared to controls, suggesting a potential link. Initial findings hint at an inverse relationship between vitamin D levels and stuttering severity. These results underscore the importance of further research to elucidate the role of vitamin D in stuttering.

Key Words: Children who stutter, stuttering, vitamin D.

Received: 19 June 2024, **Accepted:** 15 August 2024

Corresponding Author: Hanan A. Mohamed, MD, PhD, Phoniatric Unit, ORL Department, Faculty of Medicine, Assuit University, Assuit, 71515, Egypt, **Tel.:** +02001007945155, **E-mail**: dr_hanan@aun.edu.eg

ISSN: 2090-0740, 2024

INTRODUCTION

Stuttering is a complex speech problem defined by disruptions to the natural rhythm and flow of speech. These speech disruptions can include phonemes, syllables, or word repetitions, intra-phonemic disruptions, prolongations, as well as blocks during speech[1]. While stuttering is often used broadly to describe various forms of speech disruptions seen in individuals, the specific condition of stuttering encompasses intricate emotional, behavioral, physiological tension, and cognitive elements[2]. Stuttering typically emerges in children between the ages of 2 and 4, affecting around 5% of the child population. Children who stutter (CWS) face significant challenges that negatively impact their psychosocial development and communication abilities[3].

The multifaceted origins of stuttering involve genetic, neurological, environmental, and psychological factors[4]. Research from family, twin, and segregation studies strongly indicates a significant genetic predisposition to stuttering, with many individuals who stutter having relatives who also exhibit the condition. However, estimates of the heritability of developmental stuttering have shown considerable variability across different research investigations[5].

DOI: 10.21608/ejentas.2024.298118.1766

Views from a neurobiological perspective explore the brain processes that cause stuttering. Studies using functional and structural brain imaging have revealed abnormalities in areas linked to motor control and speech production[6]. CWS demonstrate variances in connectivity among brain regions. When contrasted with children who typically do not stutter, CWS show reduced functional connectivity in cortical and subcortical structures, including the supplementary motor area, thalamus, and basal ganglia, as well as the neural pathways linking them, such as dopaminergic and serotonergic circuits[7]. Also, CWS exhibits a reduction in white matter growth in different areas related to language and speech production when compared with controls[8].

While genetics and neurobiology play significant roles, environmental and psychological influences such as early life events, family dynamics, and social stigma also contribute to its development and maintenance[5]. Research suggests that over 80% of stuttering cases stem from developmental factors, although other elements such as cognitive capacity, genetic predisposition, the child's gender, and environmental influences also contribute to the onset. Furthermore, the initiation and continuation of stuttering from childhood through adulthood are believed to be impacted by linguistic, sensory, motor, and emotional factors[9].

Among the myriad factors implicated in stuttering, the emerging evidence proposes a potential relation between deficiency of vitamin D (VD) and the onset or exacerbation of stuttering symptoms. VD, acting like a prohormone that has neuroactive characteristics and is known for its crucial role in various physiological processes beyond bone health, including neurodevelopment and immune function, has a notable impact on regulating cell proliferation, differentiation, and peroxidation across different body entities, such as the brain[10].

The low serum VD effect on neurodevelopment, particularly cognitive and linguistic skills, is becoming more widely acknowledged[11]. Past research has shown that inadequate VD levels during development can result in lasting impairments in learning and memory. Additionally, reports suggest that VD deficiency might contribute to schizophrenia, autism, depression, and multiple sclerosis onset[12].

The majority of research on VD levels has concentrated on particular populations afflicted by specific illnesses and age groups, neglecting to adequately address the VD status of preschool-aged children. This oversight is troubling considering the widespread occurrence of VD deficiencies among preschoolers globally[13].

Examining this association could have substantial implications for public health. It is important to know that there are no sufficient studies currently available in the literature that have specifically investigated the relation between VD levels in preschool-aged CWS in Egypt. So, this research aims to study the association between stuttering in preschool-aged children and insufficient VD levels (specifically, 25-hydroxy cholecalciferol) in order to assign vitamin D deficiency as contributing factor for stuttering occurrence.

PATIENTS AND METHODS

Between January 2023 and December 2023, a Case-Control study was conducted at the Phoniatric unit of Aswan University hospitals on Arabic-speaking children aged 4-6 years. The study received approval from the Faculty of Medicine's Research Ethics Committee, Aswan University, on November 2022 under IRB 687/11/22. Written consent was obtained from the parents of each child after providing full information about the study. The procedures followed the 2013 Helsinki Declaration and the ethical standards of the responsible committee on human experimentation.

The participants included children with an average intelligence quotient (IQ) of 90 or above, as assessed by the Stanford-Binet test, Arabic Edition, Fifth Edition (SB5)[14]. They also had normal hearing (20–30 dB and 2-4 kHz) and average language age, measured by the Modification of the Preschool Language Scale - 4(PLS4)[15].

The study employed a 2:1 design, with controls and children who stutter (CWS) being age- and sex-matched healthy children.

G*Power 3 software was utilized to determine the sample size[16]. Based on a one-tailed test, with a 95% power and a 0.05 error probability, the calculation suggested that a sample size of 36 participants was necessary for the study design with a ratio of 2 to 1 (24 children with stuttering and 12 sex- and age-matched healthy controls). This sample size was deemed sufficient for detecting an effect size of 0.8[17].

The study excluded children who had visual or auditory impairments, a history of delayed language development, other speech disorders, or had received language or speech therapy in the past. Additionally, children with chronic, mental, neurological, endocrine, or psychiatric disorders were excluded. Those who had experienced eating disorders or had taken any medications, including VD in the three months leading up to the study, which could potentially affect VD measurements, were also excluded.

The children's demographic information (name, birth date, gender, number of siblings, clinical signs of VD deficiency, and medical history, like the last VD assessment, delayed motor development, and fracture history) is gathered from the parents. Then the children

underwent both general and vocal tract examination to rule out any organic conditions that could impact their speech.

The Arabic Stuttering Severity Index (A-SSI) was used to assess Stuttering Severity (SS) in each participant. This version, derived from the Stuttering Severity Instrument (SSI3), was standardized specifically for Arabic-speaking individuals who stutter. It has shown strong validity and reliability in previous evaluations[18]. The assessment evaluated the SS in CWS by measuring four aspects of speech behavior including frequency, naturalness, duration, and physical co-occurrences of speech. Two phoniatricians with over 15 years of experience administered the test in two distinct contexts, and the inter-judge agreement between them was documented. SS scores ranged from 0 to 19, where very mild, 20 to 22 as mild, 23 to 30 as moderate, 31 to 33 as severe, and 34 to 45 as very severe. The test was standardized for these distinctions.

Following the completion of the stuttering assessment protocol, the serum VD levels of each group were evaluated using an electrochemiluminescence binding assay. This assay is designed for use with the Cobas e411 immunoassay analyzer to quantitatively measure human serum and plasma 25-hydroxy VD in vitro, assisting in identifying VD insufficiency. Using 25-hydroxy cholecalciferol in assessing VD deficiency provides a robust and reliable method because it is widely regarded as the most reliable indicator of VD levels since it is a reflection of both food consumption and synthesis from sunlight. Its extended half-life compared to other forms of VD contributes to its stability as a marker for assessing VD levels over time. Furthermore, standardized assays for measuring 25-hydroxy VD levels ensure consistent and reliable assessment across various laboratories, enhancing the accuracy of diagnosis and monitoring of VD deficiency[19].

Blood specimens were collected, with five milliliters of venous blood extracted and placed into additive-free tubes. Within one hour of collection, serum samples were separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Subsequently, the samples were stored in separate portions at -80°C before analysis.

The classification of VD levels followed a review of recent literature: Deficiency was characterized by levels below 20ng/ml, insufficiency by levels ranging from 20 to 29ng/ml, and sufficiency by levels between 30 and 100ng/ml and potential toxicity as more than 100ng/ml[20].

Statistical analysis

Data were verified, coded by the researcher, and analyzed using IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA)[21]. Descriptive statistics: Means, standard deviations, medians, ranges, frequency, and percentages were calculated. Test of significances: Test of significances: Chi-square/Monte Carlo exact test was used to compare the difference in distribution of frequencies among different groups as appropriate. Test of normality, Shapiro-Wilk was used to test the normality of continuous variables. For continuous variables with two categories, an independent sample t-test was calculated to test the differences in mean between groups. A one-way-ANOVA test was calculated to test the mean differences of continuous data with more than two categories, post-hoc test was calculated using Bonferroni corrections for pairwise comparisons. For the assessment of agreement between raters, weighted Kappa, correlation, Cronbach's alpha, and inter-class correlation were measured. A significant p-value was considered when it was <0.05.

RESULTS

The present case-control study involved 36 preschool children, aged between 4 and 6 years, who were recruited from the outpatient clinic of the Phoniatric unit at Aswan University Hospitals. The study comprised 24 CWS and 12 healthy controls matched for sex and age, as illustrated in Figure 1 of the flow chart.

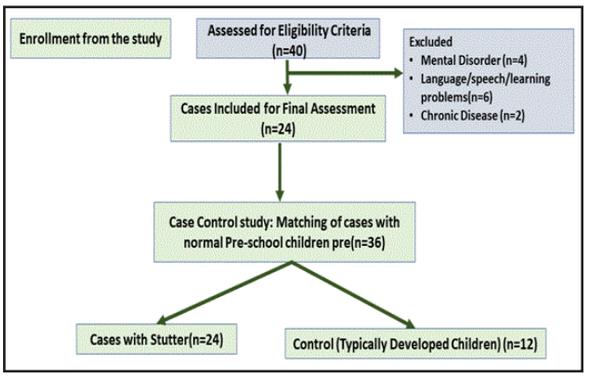

Figure 1: Flow Chart for the Total Sample.

Table 1 displays that the age distribution of the case and control groups was balanced, with stutters having a mean age of 64.33±5.1 months with no notable statistical distinction (p= 0.104). They also were matched for sex, males were predominant in both groups 79.2% of CWS and 66.7% of control children), with no notable statistical distinction (p= 0.208).

The serum VDLs were significantly lower in CWS, ranging from 8 to 28.5ng/ml, with a mean of 16.80±6.2 compared to levels ranging from 9 to 38.5ng/ml and a mean of 22.49±8.2 in the control children (p= 0.019) (Figure 2). The VD deficiency prevalence was higher in CWS (62.5%) compared to control children (41.7%). However, there was no statistically significant difference in the allocation of VD classifications among the two groups (p= 0.214), as indicated in Table 1.

The assessment of the SS revealed that CWS had A-SSI score values ranging from 15 to 26, with a mean of 21.29±2.9. Classification according to SS demonstrated that 29.2% (n= 7) nearly one-third had very mild severity, 25% (n= 6) one-quarter had mild severity, and 45.8% (n= 11) about one-half had moderate severity (Table 2).

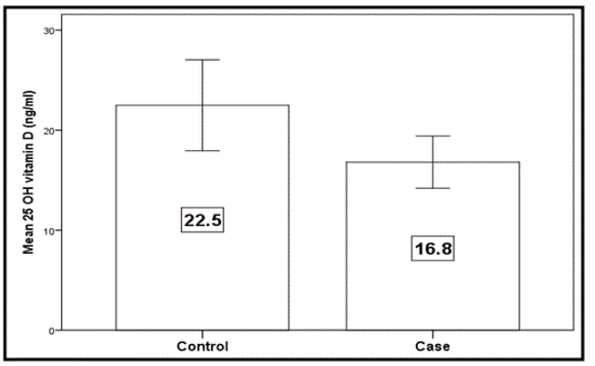

Figure 2: Difference in Mean 25-OH Vit-D level between groups.

Table 1: Correlates of children who stutter among the studied Groups:

	Control (*n*= 12)	Stutter (*n*= 24)	*P*-value
Age/months (mean±SD)	60.40±8.1	64.33±5.1	=0.104*
Sex			
• Male	8(66.7%)	19(79.2%)	=0.208**
• Female	4(33.3%)	5(20.8%)	
25 OH vitamin D (ng/ml)			
• Mean±SD	22.49±8.2	16.80±6.2	=0.019*
• Median (Range)	20.5(9–38.5)	15(8–28.5)	
25 OH vitamin D Category			
• Sufficient (>30 ng/ml)	2(16.6%)	0(0%)	=0.214***
• Insufficient (20-30 ng/ml)	5(41.7%)	9(37.5%)	
• Deficient (<20 ng/ml)	5(41.7%)	15(62.5%)	

*: Independent Sample T-test was used to compare the differences in Mean between groups; **: The Chi-square test was used to compare the frequency differences between groups; ***: The Monte Carlo exact test was used to compare the frequency differences between groups.

Table 2: Severity of stuttering according to A-SSI:

Variable	Category	*n*= 24
A-SSI	• Mean ± SD	21.29±2.9
	• Median (Range)	21(15–26)
Stuttering Severity	• Very mild	7(29.2%)
	• Mild	6(25%)
	• Moderate	11(45.8%)

There was significant-excellent agreement (weighted kappa= 0.915, $p< 0.001$) between the two raters in the evaluation of the SS levels. In other words, both raters agreed in 22(91.7%) patients and disagreed in 2(8.3%) patients i.e., 1st rater diagnosed them as mild and moderate while 2nd rater diagnosed them as slight and mild, respectively.

The reliability statistics revealed a strong correlation between two scores ($r= 0.957$, $p <0.001$), and excellent reliability (Cronbach's Alpha= 0.977, $p <0.001$ and ICC= 0.955, $p <0.001$).

The examination of the connection between A-SSI categories and VD categories among CWS showed a non-significant association ($p= 0.175$). Severe stuttering cases exhibited higher rates of VD deficiency (53.3%) compared to insufficient levels (33.3%), as outlined in Table 3. However, a statistically significant negative correlation was observed between VD levels and A-SSI score values ($r= 0.279$, $p= 0.049$), as depicted in Figure 3.

Table 3: Correlates of children who stutter among the studied Groups:

Digital Measure	Insufficient (2) (*n*= 9)	Deficient (3) (*n*= 15)	*P*-value
SSI Category			
• Very mild	4(44.4%)	3(20%)	
• Mild	2(22.2%)	4(26.7%)	0.175*
• Moderate	3(33.3%)	8(53.3%)	

*: The Monte Carlo exact test was used to compare the frequency differences between groups.

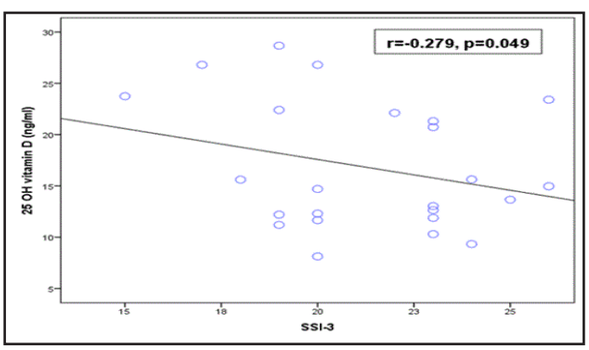

Figure 3: Correlation between 25-OH Vitamin-D Level and SSI score.

DISCUSSION

Stuttering is a communication disorder marked by disruptions in speech fluency, patients with stuttering may eventually develop involuntary motions and verbal or situational avoidance behavior. Stuttering can vary in severity and may impact social, educational, and occupational aspects of an individual's life[22].

While the precise etiology of stuttering remains multifactorial and not fully understood, emerging evidence suggests that environmental factors, including nutritional deficiencies, may play a role in its onset and severity. Among these potential factors, VD deficiency has garnered increasing attention for its purported impact on neurological development and speech fluency[23].

VD is a crucial lipid-soluble vitamin with a steroidal structure, playing essential roles in various cellular and molecular functions. Recent studies have reported that VD deficiency has been linked to disorders of memory and learning, cognitive impairment, stuttering, and the etiology of mental illnesses such as depression, schizophrenia,

autistic spectrum disorder, and multiple sclerosis. It has also been associated with cochlear hearing loss, Meniere's disease, and otosclerosis[24].

VD deficiency is a global health concern, but research focusing on VD levels in preschool-aged children remains limited. CWS face developmental challenges, highlighting the need for understanding their needs. Speech issues from stuttering may extend beyond childhood, warranting a study of its link to VD deficiency in preschoolers. Our study aimed to investigate the connection between stuttering in preschool-aged children and insufficient VD levels (specifically, 25-hydroxy cholecalciferol).

Based on this aim, we conducted a case-control study involving 36 participants aged 4-6 years, following a 2:1 design. This comprised 12 healthy subjects matched for age and sex serving as controls, and 24 participants identified as stutterers. The SS was assessed using the A-SSI instrument, leading to classifications of slight stuttering at (29.2%), mild stuttering at (25%), and moderate stuttering at (45.8%). SS Assessment inherently involves some degree of subjectivity, so, it was important to be done by two expert phoneticians to minimize the percentage of biased results. This suggests a varied degree of severity among the participants, with a significant portion falling into the moderate category.

Successful age matching between children with speech issues (CWS) and control children is crucial for minimizing potential confounding effects of age-related factors on observed differences in serum VD levels. Similarly, the lack of a statistically significant difference in sex distribution ensures that any differences in serum VD levels are less likely to be influenced by sex-related factors.

Among the participants, males exhibited a significantly higher increase in stuttering scores. Studies by (**Yairi and Ambrose**[25], **Howell and Davis**[26], and **Kang *et al.,***[27]) have indicated that gender represents a significant predisposing factor, notably elevating the occurrence of stuttering in males compared to females. This observation is consistent with findings from a specific study by **Craig *et al.,***[28] which noted a substantial male-to-female ratio in stuttering, often exceeding four to one. However, there have been reports of relatively smaller gender ratios specifically concerning SS scores[29].

The higher percentage of VD deficiency among CWS compared to control children supports the notion of an association between stuttering and VD deficiency. Although there was no statistically significant difference in the distribution of VD categories between the two groups the trend toward higher deficiency rates in the stuttering group warrants attention.

As regards the association between VD categories and A-SSI categories among CWS there was a lack of statistical significance, the association between them initially suggesting that there may not be a direct relationship between two factors. This outcome implies that, on the surface, the VD levels in the body may not be a determining factor in SS among children. The observed rates of VD deficiency and insufficiency across different severity levels of stuttering also seem to support this notion, as the proportion of severe stuttering cases with VD deficiency is higher compared to those with insufficient levels, albeit without statistical significance.

The statistically significant negative correlation between VD levels and A-SSI score values adds an interesting dimension to the analysis. This correlation indicates that as VD levels decrease, SS tends to increase. While the correlation coefficient may not be exceptionally high, the statistical significance suggests that there is indeed a relationship worthy of attention.

Consistent with earlier research conducted by **Sağiroğlu *et al.,***[30], who investigated VD levels in Turkish children with language and speech disorders, these results indicate a potential link between VD deficiency and speech disorders, including stuttering, in children. They observed that as SS increased, VD levels tended to decrease.

Following our investigation, another study conducted by **Almudhi and Gabr**[31] similarly found a notable correlation between decreased levels of VD and stuttering in children up to the age of 10. Children experiencing mild to moderate stuttering exhibited a significant decrease in VD levels compared to their healthy counterparts.

So, the negative correlation between VD levels and SS noticed in a few numbers of studies including our study, raises the possibility that inadequate VD levels could potentially have a mitigating effect on stuttering symptoms. This opens up avenues for exploring the mechanisms through which VD might influence speech fluency and motor control, potentially through its neuroprotective and neuromodulator properties.

Possible mechanisms that may suggest the VD's role in stuttering:

Research has indicated that language and speech problems including stuttering, are associated with specific biochemical, biological, and neurobiological changes affecting brain function[32].

VD receptors are distributed across various regions of the brain involved in speech and language processing such as the cerebral cortex and basal ganglia, indicating its crucial role in neurodevelopment during critical periods of brain development. As VD contributes to cell differentiation regulation, proliferation, and peroxidation, so, VD deficiency may disrupt neural circuitry related to speech production and fluency, potentially contributing to stuttering[33].

Upon administration of VD, an increase in gamma-glutamyl transpeptidase (gamma GT) levels in the brain has been noted, particularly in astrocytes and pericytes. Gamma GT is implicated in the elimination of reactive oxygen species, leading to hypotheses regarding vitamin D's role in regulating processes of detoxification in the brain[34]. This hypothesis proposes that VD enhances intracellular glutathione pools, reducing the production of harmful oxygen and nitrite species. These findings underscore the fundamental roles of gamma GT, glutathione, and VD in the astrocyte system, contributing at least partially to their neuroprotective effects[31].

VD deficiency has emerged as a potential factor contributing to changes in the structure of the brain and connectivity, mainly impacting areas involved in motor control and language processing. For instance, studies have revealed associations between VD deficiency and alterations in gray matter volume, the integrity of white matter, and connectivity patterns in specific brain regions such as the motor cortex, basal ganglia, and areas involved in speech processing. These alterations could disrupt the smooth coordination of speech-processing abilities, contributing to the development or exacerbation of stuttering[35].

Myelination is critical for the appropriate functioning of neural circuits involved in the production of speech. VD deficiency may impair myelination and synaptic plasticity in these circuits, affecting speech fluency and contributing to stuttering[36].

VD deficiency may contribute to stuttering through various mechanisms related to speech-motor control. Firstly, VD is involved in the control of synaptic plasticity and the dopaminergic system, it is involved in the regulation of dopamine and serotonin, which play roles in motor control and speech fluency. Dysregulation of these neurotransmitter systems due to VD deficiency could affect the coordination of speech-related motor movements, contributing to stuttering symptoms[37].

Additionally, optimal levels of VD are necessary for motor learning and skill acquisition, processes vital for developing fluent speech patterns. VD receptors in the hippocampus, a critical region for neuromuscular learning, may be relevant to stuttering[38]. Activation of these receptors influences synaptic plasticity and neurogenesis, important for forming and refining motor memories, including speech production. Impairment of VD levels could disrupt these processes, impacting neuromuscular learning and contributing to speech-motor difficulties observed in stuttering[39]. Moreover, the hippocampus plays an important role in sensory feedback mechanisms crucial for monitoring and adjusting speech movements in real time and might be affected by VD deficiency, potentially leading to difficulties in maintaining consistent speech patterns and fluency[40].

Although these potential mechanisms propose a feasible connection between VD deficiency and stuttering within the brain, additional research is required to clarify the exact neurobiological pathways implicated.

RECOMMENDATION

We propose implementing routine screening for serum VD levels as a standard practice for individuals with CWS. Furthermore, we support providing VD supplements to further investigate its role in stuttering and potentially enhance treatment approaches for those affected.

The strength of the study: The study focused on a specific age group (preschool children), which represents a critical period in the development of stuttering. Furthermore, the research was carried out by experienced phoniatricians and a clinical pathologist, guaranteeing expert evaluation and diagnosis within the relevant field.

The study's potential limitations include: The small sample size and demographic characteristics that might influence the extent to which the findings can be generalized. Additionally, the lack of prior research on this topic limits our ability to compare results. Furthermore, the precise association between SS and VD levels could not be definitively established due to the oversight of not including this factor in the sample size calculation.

CONCLUSION

Stuttering is a prevalent health issue, especially among preschool-aged children. This study found lower VD levels in children who stuttered compared to control children. An initial observation revealed an inverse relationship between VD levels and SS, suggesting a potential connection. Understanding these parameters could provide diagnostic benefits and aid in stuttering treatment. However, a deeper understanding of the underlying mechanisms is essential for crafting policy recommendations and public health guidelines regarding the significance of VD levels in stuttering development among preschool-aged children.

FUTUR DIRECTIONS

Conducting randomized controlled trials could evaluate the effectiveness of interventions by modifying VD levels in CWS, while longitudinal studies with a larger sample size can observe the trajectory of VD levels over time, aiding in a deeper understanding of the relationship between VD deficiencies and SS.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. N.B. Ratner, S.B. Brundage. Advances in understanding stuttering as a disorder of

language encoding. Annu Rev Linguist. 10 (2024) 127–143. https://doi.org/10.1146/annurev-linguistics-030521-044754.

2. O. Bloodstein, N. Bernstein Ratner, S.B. Brundage. A Handbook on Stuttering. San Diego, CA: Plural. 7th ed. (2021).

3. J.B. Costa, A.P. Ritto, F. Juste, FC. Assi, C.R.F. de Andrade. Risk Factors for the Development of Persistent Stuttering: What Every Pediatrician Should Know. Int J Environ Res Public Health. 19(9) (2022) 5225. https://doi.org/10.3390/ijerph19095225.

4. Bowers A. Will Brain Imaging Lead to a Translational Neuroscience of Stuttering? Perspect ASHA Spéc Interes Groups. 2023;8:943–954. doi: 10.1044/2023_persp-23-00007.

5. C. Frigerio-Domingues, D. Drayna. Genetic contributions to stuttering: the current evidence. Mol. Genet. Genomic Med. 5 (2017) 95–102.

6. S.E. Chang, E.O. Garnett, A. Etchell, H.M. Chow. Functional and neuroanatomical bases of developmental stuttering: current insights. Neuroscientist. 25(6) (2019) 566–82.

7. S.E. Chang, M. Angstadt, H.M. Chow, A.C. Etchell, E.O. Garnett, *et al*. Anomalous network architecture of the resting brain in children who stutter. J Fluency Disord. 55 (2018) 46–67.

8. E.O. Garnett, H.M. Chow, S.E. Chang. Neuroanatomical correlates of childhood stuttering: MRI indices of white and gray matter development that differentiate persistence versus recovery. J Speech Lang Hear Res. 62(8) (2019) 2986–98.

9. J.D. Anderson, L.C. Ofoe. The Role of Executive Function in Developmental Stuttering. Semin Speech Lang. 40(4) (2019) 305–319. doi: 10.1055/s-0039-1692965. Epub 2019 Jul 16. PMID: 31311055; PMCID: PMC6910129.

10. M.D. Rodgers, M.J. Mead, C.A. McWhorter, M.D. Ebeling, J.R. Shary, D.A. Newton, J.E. *et al*. Vitamin D and Child Neurodevelopment—A Post Hoc Analysis. Nutrients. 15(19) (2023) 4250.

11. M. Dennis, A.M. Hashmi. A systematic review on the role of vitamins in Stuttering. J Speech Lang Hear Res. 61(5) (2018) 1260-1272.

12. A. Mansour, A. Amer, A. Sobh, B. Zaki, T. Abou-Elsaad, Vitamin D profile in autism spectrum disorder children and its relation to the disease severity. Egyptian J Otolaryngol. 40(7) (2024) https://doi.org/10.1186/s43163-024-00573-w

13. A. Mak. The impact of vitamin D on the immunopathophysiology, disease activity, and extra-musculoskeletal manifestations of systemic lupus erythematosus. Int J Mol Sci. 19 (2018) 2355. https://doi. org/ 10. 3390/ ijms1 908235.

14. S. Faraj. Stanford-Binet Intelligence Scales (SB5), Fifth Edition. Cairo: the Anglo Egyptian Bookshop. (2010).

15. A. Abo Hassiba, S. El Sady, A. Elshobary, N. Gamal Eldin, A. Ibrahiem, A. Oweys. Standardization, Translation, and Modification of the Preschool Language Scale -4. A Doctoral Dissertation, Faculty of Medicine, Ain Shams University: Cairo, Egypt (2011).

16. F. Faul, F. Erdfelder, A.G. Lang, A. Buchner. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 39 (2007) 175-191.

17. A.R. Beauchesne, K.C. Cara, D.M. Krobath, L.P. Penkert, S.P. Shertukde, D.S. Cahoon *et al*. Vitamin D intakes and health outcomes in infants and preschool children: Summary of an evidence report. Ann Med. 54(1) (2022) 2278-2301.

18. N. Rifaie. Arabicizing and standardizing the stuttering severity instrument on the Arabic environment. Ain Shams Med J. 50 (1999) 907–914.

19. P. Pludowski, I. Takacs, M. Boyanov, Z. Belaya, C.C. Diaconu, T. Mokhort, N. *et al*. Clinical practice in the prevention, diagnosis and treatment of vitamin D deficiency: a central and eastern European expert consensus statement. Nutrients. 14(7) (2022) 1483. https://doi.org/10.3390/nu14071483.

20. M.F. Holick. The vitamin D deficiency pandemic: Approaches for diagnosis, treatment, and prevention. Rev Endocr Metab Disord. 18 (2017) 153–165. https://doi. org/ 10. 1007/ s11154-017-9424-1.

21. IBM_SPSS. Statistical Package for Social Science. Ver.24. Standard version. Copyright © SPSS Inc., 2012-2016. NY, USA. (2016).

22. S. Caughter, V. Crofts. Nurturing a resilient mindset in school-aged children who stutter. Am J Speech Lang Pathol. 27(4) (2018) 1111–23.

23. Z. Liu, S. Huang, X. Yuan, Y. Wang, Y. Liu, J. Zhou. The role of vitamin D deficiency in the development of paediatric diseases. Ann Med. 55(1) (2023) 127-135. doi: 10.1080/07853890.2022.2154381. PMID: 36495273; PMCID: PMC9744225.

24. Y. Lu, J. Li, T. Hu, G. Huang. Serum 25-hydroxy vitamin D level is associated with cognitive impairment

in people aged 65 years and older. Ann Palliat Med. 10(7) (2021) 7479–7485. doi: 10.21037/apm-21- 568. PMID: 34353037.

25. E. Yairi, N. Ambrose. Epidemiology of stuttering: 21st century advances. J Fluency Disord, 38(2) (2013) 66-87.

26. P. Howell, S. Davis. Genetic bases of stuttering: the state of the art, 2011. Folia Phoniatr et Logop. 63(3) (2011) 157-166.

27. C. Kang, S. Riazuddin, J. Mundorff, D. Krasnewich, P. Friedman, J.C. Mullikin, D. Drayna. Mutations in the lysosomal enzyme-targeting pathway and persistent stuttering. N Engl J Med. 362(8) (2010) 677-685.

28. A. Craig, Y. Tran, M. Craig, K. Peters. Epidemiology of stuttering in the communication across the entire life span. J Speech Lang Hear Res. 45 (2002) 1097–1105.

29. H. Mansson. Childhood stuttering: Incidence and development. J Fluency Disord. 25 (2000) 47–57.

30. S. Sağiroğlu. A Comparison of Vitamin D Levels in Chidren with Language and Speech Disorders and Healthy Children in the Turkish Population. Türkiye Çocuk Hast Derg. 14 (2) (2020) 158-63.

31. A. Almudhi, S. Gabr. Distinctive pattern of serum trace elements and vitamin D levels in adolescents who stutter. [Ebook] (2023) doi:10.21203/rs.3.rs-3200620/v1.

32. L. Junuzovic-Zunic, O. Sinanovic, B. Majic B. Neurogenic Stuttering: Etiology, Symptomatology, and Treatment. Med Arch. 75(6) (2021) 456–461. doi: 10.5455/medarh.2021.75.456-461. PMID: 35169374; PMCID: PMC8802677.

33. Taylor, Francis. Effects of vitamin D deficiency on neurobehavioral outcomes in children: A systematic review. Wellcome Open Res. 5(28) (2020) DOI: 10.12688/wellcomeopenres.15730.1.

34. M. Farghali, S. Ruga, V. Morsanuto, F. Uberti. Can brain health be supported by vitamin D-based supplements? A critical review. Brain sci. 10(9) (2020) 660. https://doi.org/10.3390/brainsci10090660.

35. A. Korzeczek, N.E. Neef, I. Steinmann, W. Paulus, M. Sommer. Stuttering severity relates to frontotemporal low-beta synchronization during pre-speech preparation. Clin Neurophysiol. 138 (2022) 84-96. https://doi.org/10.1016/j.clinph.2022.03.010.

36. X. Fu, G.G. Dolnikowski, W.B. Patterson, B. Dawson-Hughes, T. Zheng, M.C. Morris, *et al*. Determination of vitamin D and its metabolites in human brain using an Ultra-Pressure LC–Tandem Mass Spectra method. Curr Dev Nutr. 3(7) (2019) 74.

37. R. Landin-Romero, C.T. Liang, P.A. Monroe, Y. Higashiyama, C.E. Leyton, J.R. Hodges, *et al*. Brain changes underlying progression of speech motor programming impairment. Brain Commun. 3(3) (2021) 205. doi: 10.1093/braincomms/fcab205. PMID: 34541532; PMCID: PMC8445394.

38. V. van de Ven, L. Waldorp, I. Christoffels. Hippocampus plays a role in speech feedback processing. NeuroImage. 223 (2020) 117319. https://doi.org/10.1016/j.neuroimage.2020.117319. [ISSN 1053-8119].

39. M.S.D. Kerr, P. Sacré, K. Kahn, H.J. Park, M. Johnson, J. Lee, S. *et al*. The Role of Associative Cortices and Hippocampus during Movement Perturbations. Front Neural Circuits. 11 (2017) 26. doi: 10.3389/fncir.2017.00026. PMID: 28469563; PMCID: PMC5395558.

40. M. Al-Amin, D. Bradford, R.K.P. Sullivan, N.D. Kurniawan, Y. Moon, S.H. Han, A. *et al*. Vitamin D deficiency is associated with reduced hippocampal volume and disrupted structural connectivity in patients with mild cognitive impairment. Hum Brain Mapp. 40(2) (2019) 394-406. doi: 10.1002/hbm.24380. Epub 2018 Sep 25. PMID: 30251770; PMCID: PMC6865549.