



Folic Acid and Vitamin B12 Deficiency, Two New Findings in Pediatric Enuresis

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Abstract

Background: Enuresis is one of the most common diseases in children. Although there are several factors involved in the occurrence of this disease, the root cause of it has remained undetermined.

Objectives: Identifying various factors responsible for enuresis may enormously contribute to solving this problem. This study, therefore, aimed to determine the level of vitamin B12 and folic acid in children with enuresis in Gorgan in 2021.

Methods: In this case-control study, 43 children with primary enuresis and 99 children without enuresis, as the control group, referring to Taleghani Hospital in 2021 were included. Folic acid and vitamin B12 levels in two groups were measured and analyzed using statistical techniques.

Results: Out of all participants, 23 (53.5%) in the case group and 53 (53.5%) in the control group were male. The mean age of children in the case and control groups were 7.60 ± 3.02 and 8.93 ± 3.15 , respectively, and the two groups were not significantly different in terms of gender and age. Vitamin B12 and folic acid levels in the case group were significantly lower than those in the control group (P -value = 0.001). There was a significant difference between the case and control groups regarding the mean levels of vitamin B12 and folic acid levels based on sex and gender.

Conclusions: In sum, it was found that children with enuresis suffered from deficiency of vitamin B12 and folic acid to some extent, which may have been a factor responsible for delaying the maturation of the central nervous system and, consequently, inducing enuresis in children.

Keywords: Child, Enuresis, Folic Acid, Vitamin B12

1. Background

Enuresis is one of the most common childhood complaints, which affects about 5% - 20% of children aged five years and varies depending on the region and race (1-4).

This problem is a urinary disorder that affects both children and parents, and negatively influences the children's self-confidence and learning ability (5, 6). Since several factors are involved in the pathogenesis of enuresis and the complexity of its relevant processes, the root cause of it has not been identified yet (7-9).

Enuresis refers to urinary incontinence for at least two nights a month at an age when urinary control is already achieved, and the patient has no congenital or acquired urinary tract disorders (4). Urination in infants and young

children is involuntary up to the age 3 - 5 years, after which it becomes voluntary (10).

Enuresis is resolved spontaneously as the children grow up. Its prevalence is 15% at the age of 5, 5% at the age of 10, and 1% at the age of 15 (11). It is defined as monosymptomatic enuresis if the child does not have voiding dysfunction and also daily incontinency (4, 12).

At the age of 2 - 4 years, the child is developmentally ready to start toilet training. The expected age for voluntary urinary control at night is the age five. Girls acquire bladder control before boys. By this age, 90-95% of children are continent during the day, and 80-85% of them are continent at night. Approximately 60% of children with enuresis are boys, of which 50% have a positive family history (8, 9).

There are two types of enuresis. In primary enuresis, the child never acquires urinary control. In secondary enuresis, which accounts for 10 - 20% of patients with enuresis, the child is initially dry for a few months and then loses control of his urination (8). Primary incontinence is often associated with a family history of delayed overnight control (9). Although most children with enuresis have no psychiatric disorder, stressful conditions can exacerbate decreased bladder control (13).

Initial enuresis treatments include correcting behavioral factors such as encouragement for dryness, punishment prevention, motivational therapy, self-hypnosis, reduction of fluid intake after 7 pm, avoidance of sugar and caffeine after 4 pm, and application of some symptomatic relief drugs (8).

It should be noted that no single laboratory finding is diagnostic; however, urinary tract infection likely predispose to enuresis should be ruled out. Structural abnormalities may be evident in 3% of children with enuresis (13). Previous studies have suggested that enuresis is associated with genetic factors such as nocturnal changes in arginine vasopressin secretion, bladder dysfunction, and sleep disorder. On the other hand, delay in the maturation of the central nervous system is a significant factor contributing to the pathogenesis of primary enuresis. However, the role of vitamin B12 and folate deficiency in reducing enuresis, which is very important for developing the nervous system, is still unknown.

2. Objectives

Limited recent studies have examined the relationship between vitamin B12 and folic acid deficiency and primary enuresis as a result of delayed central nervous system maturation, and have found a significant decrease in folate and vitamin B12 levels (14-16). This study, therefore, aimed to determine the level of vitamin B12 and folic acid in children with nocturnal enuresis and children without urinary problems in Gorgan in 2021.

3. Methods

In this study case-control study, children with confirmed primary enuresis and children without enuresis referred to the pediatric clinic of Taleghani Hospital in Gorgan in 2021 were investigated.

All children with primary enuresis diagnosed and confirmed by the pediatric nephrologist were enrolled in the study. An informed written consent was obtained from all parents, and no supplementation was used in this study.

Exclusion criteria were: The lack of consent by the parents to enter the study, the presence of underlying disease (e.g., structural brain and mental disorders, seizures, and head trauma), the presence of underlying psychiatric diseases (e.g., as autism spectrum disorder, attention deficit disorder, and hyperactivity), as well as the presence of underlying renal and urinary tract diseases, urinary tract infections, adenoids, diabetes mellitus, diabetes insipidus, and bacteremia.

According to the study by Altunoluk et al. (15), the mean levels of vitamin B12 in the group of children with enuresis and the control group were 276.1 ± 93.3 and 343.6 ± 141 (pg/mL), respectively; and those of folic acid were 5.52 ± 1.89 and 9.60 ± 1.80 , respectively. The sample size was calculated with a ratio of 2.3 controls to 1 case, which was due to the significant difference in variance of vitamin B12 estimated between the two groups ($K = 2.3$) and due to the fact that the number required to estimate the mean of vitamin B12 was higher than that of folic acid. The estimated numbers of participants in the case and control groups were 43 and 99, respectively, with a confidence level of 95% and a test power of 80%.

For each patient, a checklist was used to record the patient's data (name, age, sex, serum folic acid level, and vitamin B12).

After adequate explanation of the study, a written consent was obtained from all parents of the participants. Vitamin B12 and folic acid levels were assessed in both groups of children with and without enuresis.

Then, 2 cc of blood sample was taken from patients using a foil container, and centrifuged; then the serum was separated and stored at a temperature of -20°C. At least 500 landa serum samples were required for the test. Chemiluminescence enzyme immunoassay analyzer siemens immulite 2000 was used for the measurements.

The normal level of vitamin B12 in children is 200 - 900 pg/mL, and that of folic acid is 5 - 21 ng/mL (SI: 11.3 - 47.6 nmol/L).

The data were analyzed by SPSS software version 21. Mean, frequency, and percentage were used to describe the data. Chi-square test was performed to compare the groups, and independent *t*-test and ANOVA test were used for quantitative data. The significance level in this study was considered to be less than 0.05.

4. Results

In this study, 43 children with primary enuresis were evaluated based on the diagnosis made by a pediatric nephrologist, and 99 healthy children were selected as the control group. Out of all participants, 23 (53.5%) in the case group and 53 (53.5%) in the control group were male. There

Table 1. Descriptive Data of Two Groups^a

Characteristics	Case	Control	P-Value
Sex	0.996 ^b		
Male	23 (53.5)	53 (53.5)	
Female	20 (46.5)	46 (46.5)	
Total	43 (100)	99 (100)	
Age			
Male	8.00 ± 3.43	8.45 ± 3.02	0.061 ^c
Female	7.15 ± 2.35	9.48 ± 3.24	0.095 ^c
Total	7.60 ± 3.02	8.93 ± 3.15	0.068 ^c
HB			
Male	11.82 ± 0.23	11.64 ± 0.45	0.031 ^c
Female	11.66 ± 0.39	11.45 ± 1.59	0.948 ^c
Total	11.75 ± 0.37	11.55 ± 1.13	0.095 ^c
MCV			
Male	80.70 ± 4.63	81.78 ± 3.93	0.435 ^c
Female	82.24 ± 3.78	81.70 ± 3.95	0.732 ^c
Total	81.41 ± 4.28	81.75 ± 3.92	0.652 ^c
PLT			
Male	276348 ± 70402	279226 ± 65298	0.551 ^d
Female	269450 ± 50070	262065 ± 44184	0.691 ^c
Total	273140 ± 61176	271253 ± 56858	0.850 ^c
Urine SG			
Male	1016.35 ± 3.21	1016.08 ± 2.57	0.503 ^c
Female	1014.35 ± 4.40	1016.39 ± 2.77	0.114 ^c
Total	1015.42 ± 3.90	1016.22 ± 2.66	0.517 ^c

^a Values are expressed as No. (%) or mean ± SD.^b Chi-square test^c Mann-Whitney test^d t-test

was no difference between two groups of participants in terms of gender (P-value = 0.996) (Table 1).

The mean age of children with and without enuresis were 7.60 ± 3.02 and 8.93 ± 3.15, respectively, which was indicative of age matching (P-value = 0.068) (Table 1).

There was no significant difference between the two groups in terms of mean corpuscular volume (MCV), platelet (PLT), and SG urine (Table 1).

In this study, the mean and standard deviation of serum vitamin B12 level in the group of patients with enuresis and in the control were 181.39 ± 106.64 and 277.53 ± 129.14, respectively. Mann-Whitney test was used to

Table 2. Folic Acid and Vitamin B12 Levels According to Sex and Age in Two Groups^a

Variables	Case	Control	P-Value
Sex			
Vitamin B12 (pg/mL)			
Male	187.30 ± 118.29	279.17 ± 124.96	0.01 ^b
Female	174.61 ± 94.06	275.65 ± 135.16	0.01 ^b
Total	181.39 ± 106.64	277.53 ± 129.14	0.001 ^b
Folic acid (ng/mL)			
Male	11.12 ± 7.34	18.13 ± 4.45	0.001 ^b
Female	9.32 ± 7.32	15.98 ± 3.21	0.01 ^b
Total	10.82 ± 3.38	16.6 ± 7.94	0.001 ^c
Age			
Vitamin B12 (pg/mL)			
Under 7 years	185.75 ± 106.40	335.35 ± 127.75	0.008 ^b
Over 7 years	180.40 ± 108.22	254.73 ± 123.25	0.001 ^c
Folic acid (ng/mL)			
Under 7 years	12.41 ± 6.88	18.26 ± 4.14	0.006 ^c
Over 7 years	8.46 ± 7.54	11.94 ± 3.69	0.001 ^c

^a Values are expressed as mean ± SD.^b Mann-Whitney test^c t-test

compare vitamin B12 levels in the case and control groups, which showed that there was a significant difference between the two groups (P-value = 0.001) (Table 2).

Serum levels of folic acid in the case and the control groups were 10.82 ± 3.38 and 16.60 ± 7.94, respectively.

According to the results from t-test, the level of folic acid in the group of children with enuresis was significantly lower than that in the control group (P-value = 0.001) (Table 2).

The mean of age and standard deviation of folic acid and vitamin B12 in the case and control groups showed a statistically significant difference for both under and over the age of seven years (Table 2).

There was a significant difference between case and control groups regarding the mean levels of vitamin B12 and folic acid levels based on sex (Table 2).

5. Discussion

Children with primary enuresis are unable to wake up in response to a urinary stimulus with excessive urine production at night or have decreased bladder functional capacity. In children with primary monosymptomatic enuresis, enuresis is the only symptom (17).

Several theories have been proposed to explain possible causes of enuresis, some of which include CNS maturity retardation, behavioral components, environmental factors, allergens, low bladder capacity, sleep disorders, structural abnormalities of the urinary tract, uncontrolled bladder contractions, defects in daily vasopressin secretion or prostaglandin production, and sleep apnea syndrome (18).

On the other hand, studies have shown that the function of the lower urinary tract to collect urine and urinate at a specific time depends on a specific complex neural network located at different levels of the peripheral and central nervous system. Coordination of smooth and striated muscles of the bladder and urethra is accomplished by the complex nervous system in the brain and spinal cord, the autonomic nervous system, as well as the parasympathetic pathway of the spinal bulbo-spinal reflex which plays an important role in the urination process (1, 10).

According to several studies, moreover, there is a relationship between nocturnal enuresis and developmental delays in learning disability, skeletal maturation, language development, and physical growth. In addition, some behavioral difficulties, such as hyperactivity and attention deficit may be associated with nocturnal enuresis (6).

Recent studies on behavioral neuroscience have highlighted the importance of nutrition in brain development, and reported the negative effects of nutrient deficiency on cognition and motor neuron function of the children (19). Some studies have also demonstrated that nutritional deficiencies such as vitamin B12 and folate deficiency may cause behavioral changes in addition to a delay in puberty and maturation of CNS (14-16, 19).

Nutritional deficiency of vitamin B12 is rare in children, and nonspecific symptoms include anorexia nervosa, vomiting, and neurological changes with or without hematologic disorders such as sensory impairment, impaired vision, dizziness, paresthesia, ataxia, loss of taste and smell, involuntary urination and defecation, personality disorder, vibration disturbance, memory impairment, hypotonic seizure, developmental disorder, orthostatic hypotension, and postural tachycardia. Neurophysiological and pathological findings from studies indicate axonal degeneration, which may or may not be due to demyelination (20).

The pathogenesis of enuresis has not yet been determined, but delay in the maturation of the central nervous system appears to be an important factor involved in the pathogenesis (18). Some studies have revealed that vitamin B12 is essential for maintaining healthy brain and as a vital micronutrient has a significant role in

synthesis of deoxyribonucleic acid (DNA) for neurological functions, especially in children (21, 22). One of the important roles of vitamin B12 is formation of the myelin sheath of central and peripheral nervous systems (23, 24). Delayed myelination, thinning of the corpus callosum, and atrophy of brain are the main findings shown by brain MRI of patients with vitamin B12 deficiency, which causes some neurologic symptom in children (22, 25, 26).

Folic acid is necessary to prevent neurodevelopmental defects in early embryonic phase. Folate is especially important in the early stages of brain development, and its absence may affect myelination and dendritic formation or cause inflammation (19).

According to a case report, vitamin B12 had the potential to improve enuresis in autistic patient (27).

To the best of our knowledge, there were only three studies examining the relationship among vitamin B12, folic acid deficiency, and nocturnal enuresis in children (14-16). Our study result was in line with the finding by Albayrak et al. (14) and Altunoluk et al. (15) showing that serum levels of vitamin B12 and folic acid were significantly lower in the enuresis children than in the control group ($P = 0.001$). In Keles et al.'s study, however, folic acid level in the patient group was not lower than that in control (16).

Since limited studies have explored vitamin B12 and folic acid in nocturnal enuresis, pathogenesis of these micronutrients is still unclear.

Due to the significant difference between our two study groups in terms of the mean and standard deviation levels of vitamin B12 and folic acid, it was suggested that further detailed evaluation should be conducted to investigate the role of these two vitamins in inducing enuresis in children as well as to determine their therapeutic effects.

5.1. Conclusions

In this study, it was found that children with enuresis suffered from deficiency of vitamin B12 and folic acid to some extent, which may have been a factor responsible for delaying the maturation of the central nervous system and, consequently, for inducing enuresis in children.

Supplementary Material

Supplementary material(s) is available [here](#) [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Footnotes

Authors' Contribution: Study concept and design: L. B., F. K. and A. N.; analysis and interpretation of data: L. B., M. V., and F. K.; drafting of the manuscript: L. B., F. K., M. M., and A. N.; critical revision of the manuscript for important intellectual content: L. B., F. K., and M. M.; statistical analysis: M. V.

Conflict of Interests: The authors declare that they have no conflict of interests.

Data Reproducibility: The data presented in this study are uploaded during submission as a supplementary file and are openly available for readers upon request.

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