The Relationship of Nocturnal Enuresis to Cortisol, Urea, Creatinine, and Glucose Levels

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Abstract:

Bedwetting, or nocturnal enuresis, is defined as the involuntary discharge of urine during sleep at night in the absence of central nervous system or urinary tract abnormalities in a child aged five years or more. This study was conducted on 119 children in certain areas of the Sharqat district in Salah al-Din province from the beginning of October 2022 to the end of January 2023. The concentration of urea, glucose, and creatinine in the blood serum was measured using the Enzymatic method, while cortisol concentration in the blood serum was measured using the ELISA technique.

The results indicated a significant elevation ($P \leq 0.05$) in the cortisol level in the patient group, with an average µg/l (6.34 ± 0.13) in comparison to the control group µg/l (0.08 ± 5.27). Table 2 presents the results showing a significant increase ($P \leq 0.05$) in both urea and creatinine in the patient group, with averages of ml/dl (0.83± 32.17) and ml/dl (0.02± 0.76) respectively. However, the glucose test results showed no significant difference between the patient group and the control group.

The aim of this study is to examine hormonal variables such as cortisol, urea, creatinine, and blood glucose levels.

Keywords: Nocturnal Enuresis, Bedwetting, Cortisol, Urea, Creatinine, Blood Glucose Levels.

Introduction:

Bedwetting, also known as nocturnal enuresis (NE), is defined as the involuntary discharge of urine during sleep at night in the absence of any defects in the central nervous system or urinary tract in a child aged 5 years or older. Estimates suggest that 6 million children wet their beds annually in the United States. This condition occurs in 15% of 5-year-old children, 5% of 10-year-old children, and 1% of 13-year-old children. Without treatment, 15% of children cease bedwetting annually. Generally, bedwetting diagnosis resolves spontaneously; however, 1% of these cases are resistant to all treatment methods.

Nocturnal enuresis appears in children aged between five and sixteen years across the globe, in all cultures, and across all social and economic classes. A close correlation exists between parental history and childhood enuresis. Previous studies have highlighted numerous definitions and descriptions of symptoms for involuntary urination in children and adolescents. Reported prevalence rates for these symptoms vary according to the selected definition and the age group of the children included in the study. This makes it challenging to compare various aspects of nocturnal enuresis across age groups, cultures, and regions. However, the true occurrence of nocturnal enuresis may be unknown due to underreporting because of embarrassment or cultural circumstances.

According to the current definition by the International Children's Continence Society (ICCS), "any type of wetting episode that occurs in discrete amounts during sleep is referred to as incontinence or nocturnal enuresis, regardless of the presence of accompanying daytime symptoms or any other symptoms that may be present". This definition applies from the age of five as recommended by the ICCS, the World Health Organization (WHO), and the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM V) (Austin et al., 2015).

The prevalence rate of nocturnal enuresis (NE) decreases with age during the school stage as the child transitions into adolescence. This is attributed to the natural process of spontaneous resolution as the child matures and occurs at a rate of approximately 15-20% annually so that it drops to 1-2% by the age of fifteen and until puberty. The International Children's Continence Society (ICCS) primarily categorizes it as "monosymptomatic nocturnal enuresis" (MSNE), and NE is referred to as non-monosymptomatic enuresis when accompanied by symptoms such as urinary difficulty, suprapubic pain, and daytime incontinence. Furthermore, it also delineates co-morbid conditions that are deemed related and significant in conjunction with NE, including constipation, urinary tract infections (UTIs), abdominal pain, asymptomatic bacteriuria, vesicoureteral reflux, psychological and neurological disorders, learning disorders, sleep disorders, and diabetes.

One of the causes of involuntary urination is the increase in the secretion of cortisol, a hormone produced from the middle layer of the adrenal cortex, which is released from the anterior pituitary gland due to the stress and anxiety experienced by children.

A predictive correlation was also observed between factors such as race, age, gender, birth order, level of education, parents' occupational status, family size, income, social and economic status, sleep habits, and the

frequency of NE and involuntary urination. In most cases, the cause of bedwetting is unknown. Examination by a doctor should always be the first step in ruling out any underlying diseases or conditions such as diabetes, urinary tract infections, deformities in the urethral valve in boys or the ureter in girls, and spinal cord deformities.

The aim of this study is to investigate hormonal variables such as cortisol, urea, creatinine, and blood glucose levels in the study group.

Methods:

Study Population:

This study was conducted on 119 children in some regions of the Sharqat district in the Salah al-Din province for the period from the beginning of October 2022 to the end of January 2023, to estimate the concentration of cortisol hormone, as well as concentrations of some biochemical compounds such as urea, creatinine, and random blood sugar.

Sample Collection

Blood Sample: Blood samples were collected from children, each sample containing approximately 5ml of venous blood using medical syringes. The blood was placed in tubes containing an anticoagulant substance and preserved in a box filled with ice to keep the samples fresh until the desired quantity was collected. They were then placed in tubes containing a gel and centrifuged using a Centrifuge machine at a speed of 3000 revolutions per minute for 15 minutes. This was done to separate the blood serum, which was then extracted using a Micropipette and placed in a small test tube and stored in a freezer at -20°C until the measurement of cortisol, urea, creatinine, and glucose, which our current study entails.

Biochemical Tests

Determination of Urea Concentration in Blood Serum: The urea concentration in the blood serum was estimated using the Enzymatic Method with ready-made tests from the Spanish company, Bio Systems. This method is a colorimetric one (14). Three Cuvette test tubes were prepared (Blank, Standard, and Sample). 1ml (1000 µl) of working reagent was added to each tube consecutively. Then, 10 µl of the Standard solution was added to the tube labeled as "Standard", and 10 µl of blood serum was added to the tube labeled as "Sample". These were well mixed and placed at room temperature (37Cº) for 5 minutes. After this, 1ml (1000 µl) of R3 was added, and after mixing, they were incubated at a temperature of 37Cº. The device was then zeroed on the

Blank tube, and readings were taken for the Standard and Sample at a wavelength of 600 nm.

Determination of Creatinine Concentration in Blood Serum:

The creatinine concentration in the blood serum was estimated using ready-made tests from the French company, Bio Labo. This method is a colorimetric one (16). Two Cuvette test tubes were prepared (Blank, Standard, and Sample). 1ml (1000µl) of working reagent was added to each tube consecutively. Then, 100µl of Demineralised water was added to the tube labeled "Blank", 100µl of the "Standard" to the tube labeled "Standard", and 100µl of the "Sample" to the tube labeled "Sample". The readings were taken on the device at a wavelength of 490nm. Firstly, the device was zeroed after placing the Blank tube (containing the distilled water). Then, the reading of the Standard tube was taken and the reaction time (30 seconds) was recorded. The first reading was taken at the 30-second mark of the first minute, and the second reading was taken at the two-minute-and-30-seconds mark. The same method was used to read the **patient's sample, recording both readings.**

Determination of glucose concentration in blood serum

The concentration of glucose in blood serum was determined based on the enzymatic method using ready-made assays from Randox, an Irish company. Three Cuvette test tubes were prepared (Blank, Standard, and Sample). To each tube, 1ml (1000µl) of the working reagent was added consecutively. Then, 10µl of the Standard solution was added to the tube labeled Standard and 10µl of blood serum was added to the tube labeled Sample. They were mixed well and then incubated at a temperature of 37C^{o} for 10 minutes. Afterwards, the device was zeroed on the Blank tube in the Spectrophotometer and the readings were taken for both the Standard and the Sample at a wavelength of 550 nm.

Hormonal tests

Estimation of the concentration of cortisone hormone in the blood serum

An assay kit from the Chinese company Sunlong Biotech was used for this test. This ELISA kit uses the Sandwich –ELISA method. The microelisastripplate, pre-coated with antibodies specific for Cortisone, was prepared. Standards or samples were added to the wells and combined with the specific antibody, then the Horseradish Peroxidase (HRP) enzyme conjugated with the specific antibody for Cortisone was added to each well in the plate and incubated. The free compounds were then washed away. A solution of Tetramethylbenzidine (TMP) was added to each well containing Cortisone and HRP conjugated with the anti-

Cortisone antibodies, which will turn blue and then to yellow after adding the stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450nm. The OD values are proportional to the concentrations of Cortisone, and the concentration of Cortisone in the samples can be calculated by comparing the optical density of the samples with the standard curve.

Five tubes were used for the measurement. 300µl of the Standard solution was added to tube 1, then 150µl of the Standard diluent was added after mixing. 300µl of the resulting solution was taken after mixing and added to tube 2. Then 150µl of the Standard diluent was added to it. Then the resulting solution after mixing from tube 2 was taken (150µl) and added to tube 3. Another 150µl of the Standard diluent was added to it. The resulting solution after mixing from tube 3 was taken (150µl) and added to tube 4. Another 150µl of the Standard diluent was added to it. Then the resulting solution after mixing from tube 4 was taken and added to tube 5. After dilution, the concentrations in each tube were 15pg/ml, 10pg/ml, 5pg/ml, 2.5pg/ml, and 1.25pg/ml respectively and the total volume (50µl) of the resulting solution after mixing, which will be added to the MicroelisaStripplate.

Statistical analysis:

A statistical analysis of the current study data was conducted using the Chi-square test using SPSS, and the current study data was presented using tables and graphs.

Results: The results in Table (1) showed the distribution of the study groups according to the disease incidence, with the control group consisting of 29 individuals (24.4%), and the patient group having 90 individuals (75.6%). The total number of individuals was 119 (100%). Table (2) showed the levels of cortisone hormone, urea, creatinine, and glucose in the blood for both patients and control. The level of cortisone in patients was (6.34±0.13)µg/l and in control was (5.27±0.08)µg/l. The level of urea in patients was (32.17±0.83)mg/dl and in control was (28.16±1.66)mg/dl. The level of creatinine in patients was (0.76±0.02)mg/dl and in control was (0.65±0.04)mg/dl. The level of glucose in patients was (86.74±1.21)mg/dl and in control was (83.48±1.20)mg/dl.

Table 1: Distribution of study groups by disease incidence

Study Groups	Number (%)
Control	29 (24.4%)
Patients	90 (75.6%)
Total	**119 (100%)**
p value	0.0021**

The results shown in Table 2 indicate a significant difference ($P \leq 0.05$) in cortisone levels between the patient group (6.34±0.13)µg/l and the control group (5.27±0.08)µg/l, with higher levels observed in the patient group.

Table 2: Biochemical attributes of the study groups (Patients and Control)

Study Groups	Cortisol (µg/l)	Urea (mg/dl)	Creatinine (mg/dl)	R.B.S (mg/dl)
Control	0.08 ± 5.27 b	1.66± 28.16 b	0.04± 0.65 b	1.20± 83.48 a
Patients	0.13 ± 6.34 a	0.83± 32.17 a	0.02± 0.76 a	1.21± 86.74 a
p value	0.0001**	0.0224*	0.0213*	0.1787

'*','**' : significant

Results shown in Table 2 illustrate a statistically significant increase ($P \leq 0.05$) in urea and creatinine in the patient group with averages of 0.83± 32.17 mg/dl and 0.02± 0.76 mg/dl, respectively, compared with the control group. No significant difference was observed in glucose levels between the patient group and the control group. The results also indicate a statistically significant increase ($P \leq 0.05$) in cortisol concentration, with an average of 0.13 ± 6.34 µg/l, as depicted in Figures 1, 2, 3, and 4.

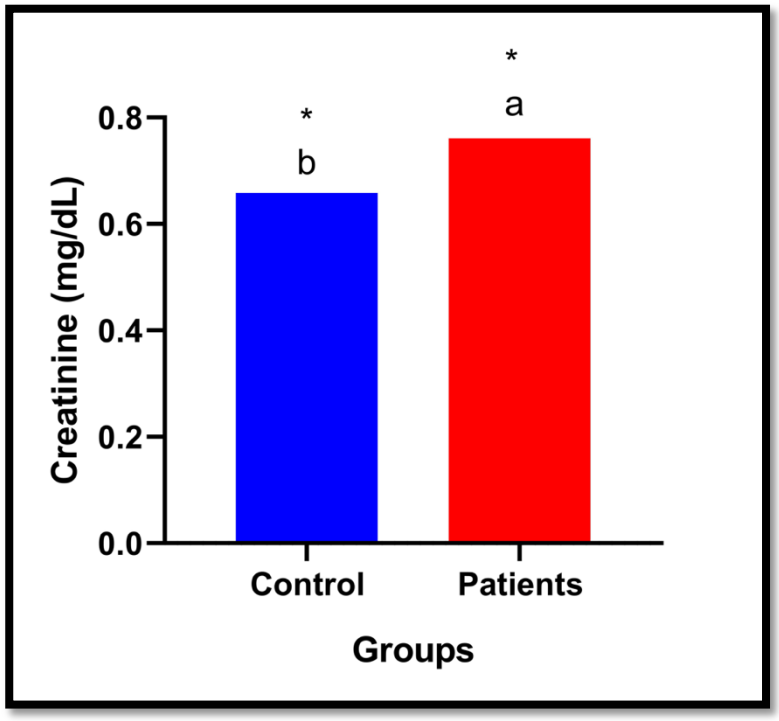

Figure 2: Creatinine Level in Blood for Both the Patient Group and Control Group.

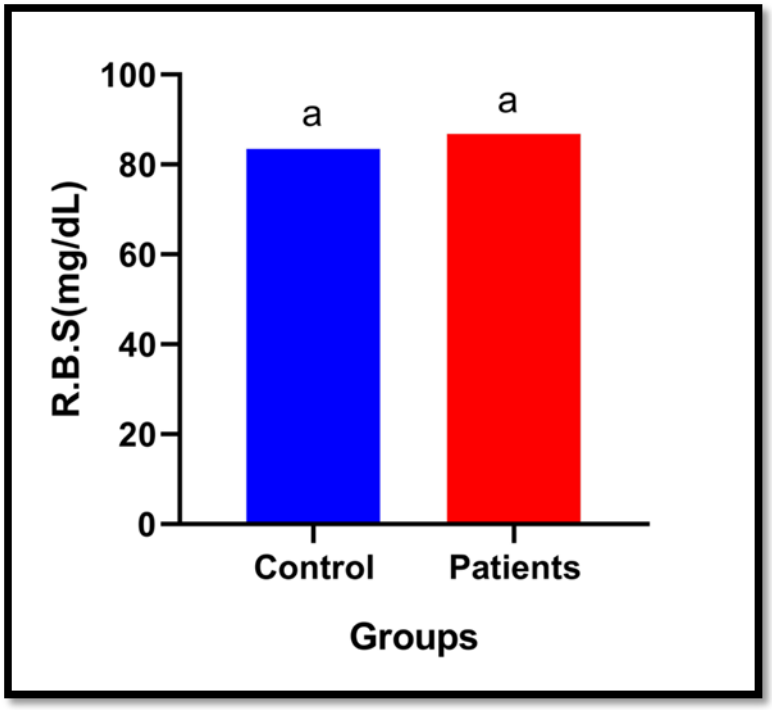

Figure 3: Blood Sugar Level in Both the Patient Group and Control Group.

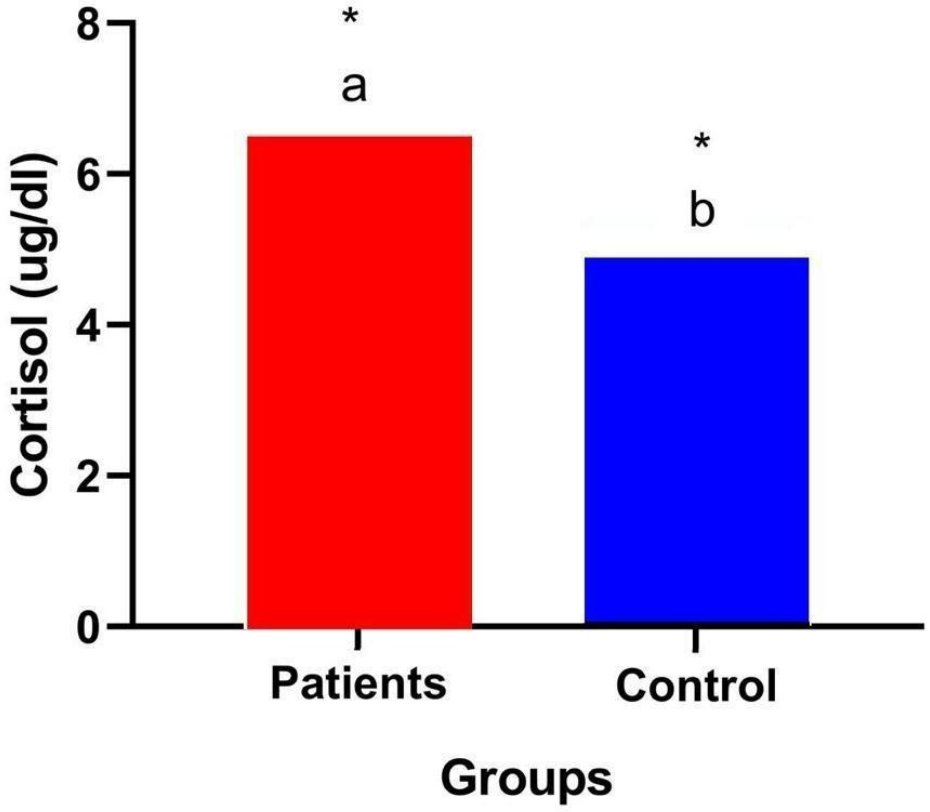

Figure 4: Cortisol Level in Both Patient Group and Control Group.

Discussion

Physiologically, cortisol, also known as the hormone released in times of extreme stress, has a negative effect on the pituitary gland, which can alter the synthesis and secretion of the antidiuretic hormone (ADH). There is also evidence that the stress hormone cortisol suppresses the release of the antidiuretic hormone, leading to urine retention and thus an increase in the volume of urine in the bladder {1}.

Because of the contradiction between nocturnal urine production and bladder capacity, the bladder may easily fill up at night, leading to the child waking up to urinate or in children who have difficulty waking up {2}. Under normal circumstances, the secretion of nocturnal vasopressin is higher than it is during the day. This leads to a 50% reduction in urine production during the night.{4}

As the results have shown, there was a significant increase ($P \leq 0.05$) in both urea and creatinine in the patient group, compared with the healthy control group. This result contrasts with findings in a Turkish study {8}, where no significant statistical relationship was found between levels of urea and creatinine at a probability level ($p > 0.05$) {8}.

The results of our study also did not coincide with the results of the study conducted by researchers {9}, where the concentration of creatinine in the blood and the excretion of urinary creatinine in the duration (g/24 h) did not differ significantly between patients with incontinence and the healthy group at a probability level ($p = 0.692$) {9}. This also differs from the results {10}, where there were no significant differences in urea and creatinine ratios between the patient group and the control group.

As for the glucose test, the results did not record any significant differences between the patient group and the control group.

Desmopressin, typically associated with diabetes insipidus, is a synthetic analogue of vasopressin (ADH) with a more pronounced anti-diuretic effect. It is the preferred treatment in the alternative therapy for diabetes insipidus, as it inhibits urine production during the day and night. Vasopressin, a neurohypophysial hormone, is released when plasma osmolarity increases and blood pressure decreases.

Diabetes insipidus, as a result of vasopressin deficiency, is characterized by decreased reabsorption of water from the collecting tubules of the kidneys and patients suffer from polydipsia and polyuria {24}. Some children may experience increased urination with signs of diabetes, which may be diabetes insipidus due to decreased levels of the antidiuretic hormone, as shown in the results of our current study.

Many children who suffer from conditions such as constipation, delayed growth, sleep problems, and learning difficulties do not have enuresis. On the other hand, there are many children who suffer from these conditions who also have enuresis and wet their beds, as found in our study. These children may be prone to enuresis due to factors such as genetic and familial characteristics, household circumstances, or any shared disease conditions. These children suffer from additional morbidity due to undiagnosed and untreated enuresis, causing distress and inconvenience for both the parents and the child as a result {6;7}.

Constipation is considered one of the causes of enuresis, which may be due to the fact that the filled rectum presses on the bladder, causing overactive detrusor. A child with a neurogenic bladder will certainly wet his bed, but it is possible that enuresis is not the only symptom. Among urinary tract malformations that may cause enuresis, urethral valves deserve special mention. These boys who suffer from urethral valve malformation not only have disturbed bladder function, but they also have tubular kidney damage leading to urea-free loops of urine, increased intravesical pressure, and further renal damage {2}.

Conclusions:

1. The role of cortisol and its impact on the synthesis and secretion of the anti-diuretic hormone (ADH) underlines the complexity of urinary control mechanisms. This highlights the need for comprehensive physiological understanding when approaching related medical conditions.

2. There is a notable link between nocturnal enuresis and the levels of vasopressin (ADH) in the body. Conditions like diabetes insipidus, associated with vasopressin deficiency, can lead to symptoms such as increased nighttime urination and bedwetting in children.

3. The impact of other health conditions, such as constipation and delayed growth, on the incidence of enuresis demonstrates that a multidisciplinary approach can be beneficial in managing this condition.

4. Urethral valve malformations and their consequent renal and bladder disturbances underline the importance of thorough diagnostic procedures when investigating causes of enuresis in children.

Recommendations:

1. For children experiencing symptoms of increased nocturnal urination or enuresis, screening for conditions such as diabetes insipidus, constipation, and other physiological factors should be considered.

2. In patients with diagnosed diabetes insipidus, treatment options like Desmopressin can be explored to manage symptoms of the disease and improve the quality of life.

3. For patients with constipation and symptoms of enuresis, management of bowel function should be considered as a part of the treatment plan for enuresis.

4. In cases where enuresis is persistent and no apparent physiological cause is found, potential genetic and familial factors, as well as shared disease conditions, should be explored.

5. Education of families dealing with enuresis can be beneficial, providing understanding of the condition, its potential causes, and methods for managing it, thus reducing distress for both the child and parents.

6. Further research should be encouraged in the area of urological conditions in children, specifically focusing on uncovering novel links and potential treatments.

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