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Anion Gap and Electrolytes Disturbance in Iraqi Children With Celiac Disease

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Abstract

Background:**C**eliac disease is a genetic autoimmune disorder; children with celiac disease may have malabsorption, electrolytes and acid-base disturbance. Electrolytes like (Sodium, Potassium, and Chloride) have an essential role in maintaining blood pressure. At the same time,bicarbonate and anion gap are the best ways to measure the body's acid-base balance.

Objective:The study aims to measure electrolytes (Sodium, Potassium,and Chloride) to determine the electrolyte disturbance and to measure the Bicarbonate and anion gap to determine the acid-base imbalance in the celiac disease group compared to the control group.

Methods:

Fifty children have been included in this study (twenty untreated celiac disease and thirty control children). Three milliliters of venous blood samples were taken, Gold-top serum separator tubes were used to obtain the serum, then Sodium, Potassium, Chloride, and Bicarbonate were measured by kinetic method, and the anion gap was calculated using the equation:

Anion gap=[(Sodium +Potassium) – (Chloride +Bicarbonate)]

Results: Patients with untreated celiac disease suffer from severe hyponatremia with mild hypochloremia (p values < 0.001, 0.023), respectively. However,neither Potassium andBicarbonate concentrations nor the Anion gap have been affected.

Conclusions: Acid-base imbalance and electrolyte disturbance didn't usually occur in children with celiac disease who suffer from mild symptoms, while Sodium and Chloride concentrations decreased due to their loss through diarrhea.

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1. Introduction

Celiac disease (CD) is a genetic disease and an autoimmune disorder occurring from an immune reaction to eating gluten; this reaction occurs in the small intestine, and over time it leads to inflammation that damages the small intestine's lining, which causes malabsorption and impaired digestion of some nutrients [1]. CD can be diagnosed by Anti –Gliadin anti body(IgA ,IgG) ,Anti-Tissue trans glutaminase (IgA , IgG), and EMA (anti-endomysial antibodies)-IgA [2]

The small intestine lining biopsy confirmation is the most accurate test; the mucosa histological examination shows subtotal villous atrophy that may lead to malabsorption , crypt hyperplasia, and abnormal thickness of the mucosa [3] CD symptoms include weight loss, chronic diarrhea, abdominal distension, and high aminotransferase levels[4]**,** patients with CD have malabsorption , this occur due to mucosal damage after gluten ingestion, CD complications may include electrolyte imbalance, anemia and osteoporosis The effective treatment for CD is a lifelong strict gluten-free diet [5]

Anion gap

The anion gap (AG) is a value calculated from the difference between the sum of measured cations (Sodium $Na^+$and Potassium K^+) subtracted from anions sum(Chloride Cl^- and BicarbonateHCO3-) in serum or plasma,and it is calculated in the equation

The anion gap = [$(Na^+ + K^+)$ - $(Cl^- + HCO3-)$]

It can be normal, low, or high; the normal value for serum AG is between 8 – 16 mmol L^{-1}[6]. A high anion gapcommonly occurs in hospitalized patients. A high serum anion gap indicates metabolic acidosis due to decreased excretion or overproduction of acid or severe volume depletion.[7]

Enteropathic *Escherichia coli* and other bacteria and viruses that cause diarrhea in infants and young children may lead to metabolic acidosis and hypokalaemia when severe [8], while low Anion Gap is a relatively infrequent occurrence.Disorders which are associated with low AG are Hyponatremia, hyperchloremicacidosis, and intestinal obstruction [9]

Sodium

Sodium Na^+,is an essential cation present in the extracellular fluid. The human body plays an essential role in muscle contraction, glucose absorption, and osmoregulation. Na^+ ions are used in opposition to potassium ions to build up an electrostatic charge on cell membranes,allowing transmission of nerve impulses.

Hyponatremia is defined as a decrease in the serum Na^+ concentration to a level below 135 mmol L^{-1} . It may occur in kidney and heart problems, chronic and severe vomiting or diarrhea,anddehydration [10]

Potassium

Potassium K^+ is the main cation in the intracellular fluid; its functions in the body are acid-base regulation, maintaining cellular osmolarity, and regulating muscle and cardiac functions. It has an antihypertensive effect through promoting sodium excretion [11].

Chloride

Chloride Cl^- is the main anion of extracellular fluid (ECF). The electrolyte concentration in the ECF is assumed to be constant, and any change in electrolyte concentrations will lead to fluid shifts.

Cl^- ion is important in the maintenance of blood pressure and acid-base balance.

Cl^- level in the blood rises and falls along with Na^+ level, and it's indirectly regulated by the hormone Aldosterone, which regulates the amount of Na^+ in the blood.

The level of Cl^- in the blood is also related to the bicarbonate HCO_3^- level; when it's decreases, the amount of Cl^- increases typically, and vice versa [12]

Bicarbonate

Bicarbonate HCO3-is the second abundant anion in the blood after Cl^-. It's result from a chemical reaction between carbon Dioxide $CO_2$with waterH_2O, and the action of an enzyme called Carbonic Anhydrase through the following reactions:

$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow$ HCO3-+ H^+

HCO_3^-concentration is regulated by renal compensation; its regulatesHCO3-concentration by filtering out excess or retaining it in low concentrations [13]

High concentration of HCO3-indicates metabolic alkalosis (high level of arterial blood pH more than 7.4); this could occur in gastric losses through vomiting and/ordiarrheaand in otherstates such as congenital chloridorrhea and renal insufficiency

While lowconcentration of HCO_3^- indicates metabolic acidosis (low level of arterial blood pH less than 7.4). Many causes may lead to metabolic acidosis, such as gastrointestinal loss of HCO_3^- through diarrhea, Diabetic ketoacidosis, andlactic acidosis[14]

The study aims to measure electrolytes (Sodium, Potassium, and Chloride) to determine the electrolyte disturbance and to measure Bicarbonate and anion gap to determine the acid-base imbalance in the celiac disease group compared to the control group.

2. Research Method

This research was a case-control study. It was conducted in the Gastroenterology and Hepatology Center (Medical City),Al Imamain Al-Kadhimen Medical City (PediatricDepartment), and Central Public Health Laboratory. The Ethical Committee of College of medicine\ AL-Nahrain University approved the study protocol. The ethical clearance had been taken from the review board for the medical research committee /Al-NahrainUniversity .

Fifty children were included in this study; they were divided into two groups (twenty children with untreated Celiac disease and thirty control children who were admitted to the hospital without chronic diarrhea symptoms). A questionnaire form took the information after obtaining legal consent from the children's parents.

- In the Celiac disease group, children aged between (2-13) years, these patients were admitted to the hospital because they were suffering from episodes of vomiting after meals, loss of appetite, abdominaldistension, recurrent abdominal pain, nausea, andchronic diarrhea (14 days or more)with significant weight loss.

Common extra-intestinal manifestations are failure to thrive, short stature, chronic anemia, osteoporosis, dental enamel defect and chronic fatigue.

They were previously diagnosed by measuring their anti–Gliadin, anti-body (IgA, IgG), and anti-Tissue trans glutaminase (IgA, IgG) in Al- Imamain Al-Kadhimen medical city and in the Central Public Health Laboratory (Immunity department) which were positive, and by duodenal biopsy at the Gastroenterology and Hepatology center that shows villous atrophy with crypt hyperplasia and increased intra-epithelial lymphocytes, these tests confirmed these patients had CD.

All the patients who had been taken were not yet treated with a gluten-free diet. In the control group, the children were either admitted to the hospital without having CD or chronic diarrhea or coming to the hospital for routine checkups. Their ages were between (2-17) years.

The Exclusions criteria were: children below two years, children with tonics, children with renal disease, and children who had recent blood transfusions within the last month.

Blood Sample Collection and Storage:

Three milliliters (3 ml) of venous blood samples were taken,and Gold-top serum separator tubes were used. They contained serum gel separator and clot activator. Blood was centrifuged for 10 minutes at 3500 rpm using(Centrifuge H- 19 F), and the serum was divided into 4 tubes and frozen at-20º C to measure Sodium, Potassium, Chloride, and Bicarbonate.

2.1-Determination of Sodium

Procedure:

The kit used wasobtained from**(**Spectrum company / Germany**)**

Precipitation Step

Three plane tubes were used and marked as (Reagent Blank tube, standard tube, and sample tube) and pipette to it (by using Micro pipette Slamed (5-50) µl and (100-1000) µl):

	Reagent Blank tube	Standard tube	Sample tube
Reagent R1(Magnesium acetate 140 mmol/L, Uranyl acetate 19.0 mmol/L)	1 ml	1 ml	1 ml
Standard (Sodium 150 mmol/L)	.....	20 µl	.....
Sample	.....	.....	20 µl
Distilled water	20 µl	.....	.....

The tubes were sealed and protected from light during the whole procedure; then, they were mixed thoroughly by mixer (Karl Kolb) and left to stand for 5 minutes, then shaken for 30 second by a MS2 mini shaker and left to stand for 30 minutes.After that, they were centrifuged for 10 minutes by centrifuge.

Reaction Step:

Pipette into cuvette

	Reagent Blank (RBL)	Standard	Sample
Supernatant	20 µl	20 µl	20 µl
Reagent R2(Ammonia 550 mmol/L , Ammonium thioglycolate 550 mmol/L)	1 ml	1 ml	1 ml

The tubes were mixed by mixer(Karl Kolb) and incubated for 3 minutes at 37ºC in a (memrt) water bath; then the absorbance of regent blank (ARBL), the absorbance of standard (A standard), and the absorbance of the sample (A sample) were read against water (zero buttons was pressed after reading the absorbance of water) at wavelength 405 nm.

Calculation:

ΔA's was calculated as follows:

ΔAsample = (ARBL - Asample)

ΔAstandard = (ARBL - Astandard)

Serum Sodium concentration (mmol/L) =(ΔA Sample /ΔA Standard)x 150

Serum expected values:135 – 150 mmol /L

Sodium was measured by using a spectrophotometer Cecil CE 2031 by setting first time (0 minutes) and end time (3 minutes) and setting the factor 1; this test linearity is 300 mmol /L

2.2- Determination of Potassium

Procedure:

The kit used was purchased from (Spectrum Company / Germany)

Three planetubes were prepared, marked as (Reagent Blank tube, standard tube, and sample tube) and pipette to it (by using Micro pipette Slamed (5-50) µl and (100-1000) µl):

	Reagent Blank tube	Standard tube	Sample tube
Reagent R (NaOH 0.50 mol/L , TPB-Na 240 mmol L^{-1})	1mL	1mL	1mL
Standard (Potassium 5 mmol L^{-1})	.....	20 µL	.....
Sample	.....	.....	20 µL

These tubes were mixed with a(Karl Kolb) mixer for 3 minutes and incubated for 3 minutes at 37 ºC in (memmrt) water bath; then the absorbance of the sample (Asample) and the absorbance of standard (Astandard) against the absorbcnse of blank were read (zero button was pressed after reading the blank absorbance) at a wavelength:578 nm

Calculation:

Serum Potassium Concentration. (mmol/L) = (A Sample /A Standard) x 5

Expected Values of Potassium in serum: 3.6 - 5.5 mmol /L

Potassium was measured using a spectrophotometer Cecil CE 2031by setting first time (0 minutes) and end time (3 minutes) and set the factor 1; this test linearity is 10 mmol /L

2.3- Determination of Chloride

Procedure:

The kit used was purchased from (Biolabo Company / France)

Three planetubes were used and marked as(Reagent Blank tube, standard tube, and sample tube) and pipette to it (by using Micro pipette Slamed (5-50) µl and (100-1000) µl):

	Reagent Blank tube	Standard tube	Sample tube
Reagent(Mercuric Thiocyanate 1.33 mmol L^{-1})	1 ml	1 ml	1 ml
Deionized water	10 µL	……	……
Standard(Chloride 100 mmol L^{-1})	……	10 µL	……
Sample	……	……	10 µL

These tubes were mixed by (Karl Kolb) mixer and left to stand for 5 minutes 37 ºC in a (memrt) water bath,. The absorbance was recorded at 500 nm against the absorbance of reagent blank.

Calculation:

Concentration of Chloride = (A sample / A standard)x standard concentration.

The Expected value is :98 – 107 mmol L^{-1}

And the reaction is linear between 70-140 mmol L^{-1}

Chloride was measured by using a spectrophotometer Cecil CE1021

2.4- Determination of Bicarbonate

Procedure:

The kit used was purchasedfrom (Roche Company / Germany)

	volume
R1(Phosphoenolpyruvate ≥40mmol/L, NADH analog ≥2 mmol L^{-1}, MDH ≥314.3 µkat L^{-1}, PEPC ≥ 30.8 µkat L^{-1})	50µL
Sample	2 µL
Diluents (H_2O)	130µL
Total volume	182µL

Bicarbonate was measured using an auto-analyzer Cobas C111 at Al Imamain Al-KadhimenMedical City(dialysiscenter).

Application for serum and plasma Cobac c111test definition:

Measuring Mode: Absorbance,calculation mode: kinetic, reaction direction: decrease, wavelength: 409-512 nm, unit mmol / L, The Cobas c111 analyzer automatically calculates the analyte concentration of each sample

Expected Values of Bicarbonate in serum: 22 – 29 mmol/ L

2.5- Calculation of Anion Gap

The anion gap calculation had been done by using the equation

Anion gap = [$(Na^+ + K^+) – (HCO3- + Cl^-)$]

Statistical analysis

Data were analyzed by using statistical packages of SPSS 20 (statistical packages for social sciences-version 20) and Microsoft Excel 2013. All data had been presented as a mean ± sd. Statistical comparisons of continuous variables of the study groups were performed by independent t-test.

Linear correlation was used between two variables to show the possible causal effect relationship between these parameters by a Spearman correlation coefficient

The receiver operating characteristic curve (ROC) was used to define the diagnostic value, the best cut-off points, specificity, sensitivity, and the area under the curve were determined at the optimum cut off value for that test. For all tests; p-value < 0.05 was as considered statistically significant, and p-value <0.001 was considered as highly significant.

3. Results and Discussion

The current study included fifty children, and they were twenty children with untreated Celiac disease, while the control group was thirty children.

Sodium, potassium, chloride, bicarbonate, and anion gap were measured for all the study groups.

Table (1): Comparison of Sodium, Potassium, Chloride, Bicarbonate, and anion gap between the control group and celiac disease group by independent test

	$S.Na^+$ mmol L^{-1}	**S. K^+ mmol L^{-1}**	**$S.Cl^-$ mmol L^{-1}**	**S. HCO3- mmol L^{-1}**	**Anion gap mmol L^{-1}**
Control (n = 30)					
mean±SD	137.5±1.87	4.26±0.42	104.33±2.13	24.25±1.59	12.91±2.43
Celiac disease (n = 20)					
mean±SD	130.55±2.46**	4.05±0.27	102.7±2.6*	23.75±1.61	12.13±3.35
P	< 0.001	0.059	0.023	0.285	0.347

* Comparison with control *P* value < 0.05, ** Comparison with control *P* value < 0.001

The untreated Celiac disease patients show a highly significant hyponatremia (P value <0.001), and its concentration was(130.55mmol/ L) with a slightly significant hypochloremia (p=0.023) and its concentration was (102.7mmol/ L). No significant changes were found in the mean values of Potassium, Bicarbonate, and anion gap when compared to the control group (4.05, 23.75and12.13mmol/ L),respectively. Their p-values were all more than 0.05, as shown in Table (1); this reflects that no acid–base disturbance occurred in these patients.

Table (2): Sensitivity specificity of Bicarbonate and anion gap in celiac disease group

	Specificity	**Sensitivity**	**Area under curve**	**P value**
HCO_3mmol/ L	80 %	25 %	0.587	0.303
Anion gap mmol / L	55 %	53.3 %	0.582	0.332

Table (2) revealsthat there is low sensitivity (25%) of Bicarbonate with high Specificity (80%) for indication of acid-base disturbance in untreated CD patients, while the AG is more sensitive (53.3%) to indicate the acid-base disturbance.

In discussion,malabsorption, malnutrition, electrolyte disturbance, and acid-base imbalance may occur in children with chronic diarrhea of different causes. In this study, children with untreated celiac disease were takenas the patient group.

Electrolytes imbalances were found in patients with CD which is one an evidences of malnutrition [15]

The pathogenesis of hyponatremia in diarrhea is due to a combination of loss Na^+, $Cl^-$and water, but water retention may compensate for the volume depletion.[16]

The diarrhea that occurs due to celiac disease may be associated with T cell-mediated gut inflammation. In contrast, the activation of T cells may increase the permeability of the gut, which leads to a decrease the activity of Na+/K+-ATPase, and these differences could lead to moderate increase in the stool volume, but electrolyte disturbance as well as acid-base imbalance rarely occurs in CD patients. The (HCO3−) and (K+) concentrationsare usually normal.[17]

In our study, K^+ and Bicarbonate were normal in the CD group. Metabolic acidosis and severe metabolic disturbance include hypokalaemia. This is an indication of Celiac Crisis, which is a rare complication of CD and requires hospitalization. [18]

Celiac crisis is a rare presentation of CD; it includes acute and severe symptoms that could lead to fatal consequences. It is more frequent in pediatrics, where their ages are less than two years, and it was rarely described in adults. [19]. Its characterized clinically by dehydration, diarrhea, metabolic acidosis, hyponatremia, hypokalemia,and electrolyte disturbances significantly.[20]

The patients in this study did not have a celiac crisis. They had mild symptoms, so there had mild electrolytes abnormalities with no acid-base abnormalities**.**

4. Conclusion

Children with celiac disease may suffer from sever hyponatremia with slight hypochloremia due to their loss through diarrhea, but electrolyte disturbance and acid-base imbalance may occur only in celiac crisis, which is a rare, severe and fatal feature of celiac disease.

5. Conflict of interest

Conflict of interest: there are no conflict of interest .

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الفجوة الانيونية و أضطراب الالكتروليتات لدى الاطفال العراقيين المصابين بالداء البطني

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المستخلص :

مرض الداء البطني (حساسية القمح) هو اضطراب المناعة الذاتية الوراثي، الأطفال الذين يعانون من مرض حساسية القمح قد يعانون من سوء الامتصاص واضطراب الالكتروليتات واضطراب حامضية – قاعدية الجسم.. تلعب الإلكتروليتات مثل (الصوديوم والبوتاسيوم والكلوريد) دورًا مهمًا في الالتحام الأسموزي والحفاظ على ضغط الدم، بينما تعد البيكربونات والفجوة الأنيونية من أفضل الطرق لقياس التوازن الحمضي - القاعدي في الجسم.

الهدف من الدراسة هو قياس الالكتروليتات (الصوديوم والبوتاسيوم والكلوريد) لتحديد اضطراب الكهارل (الالكتروليتات)وقياس البيكربونات و الفجوة الأنيونية لتحديد الخلل الحمضي القاعدي في مجموعة المصابين ب مرض الداء البطني الغير معالجين ومقارنتها بمجموعة السيطرة.

طريقة العمل :

اشتملت هذه الدراسة على خمسين طفلاً (عشرون طفلاً من مرضى الداء البطني غير المعالجين وثلاثين طفلاً للمقارنة في مجموعة السيطرة)، تم أخذ ثلاثة ملليلتر من عينات الدم الوريدي، وتم استخدام أنبوب فاصل للمصل ذو قمة ذهبية للحصول على المصل، تم قياس تراكيز كل من الصوديوم والبوتاسيوم والكلوريد والبيكربونات بواسطة التقنية التفاعلية الحركية وتم قياس الفجوة الأنيونية من خلال تطبيق المعادلة التالية:-

الفجوة الأنيونية=[(صوديوم + بوتاسيوم) – (كلوريد + بيكربونات)]

النتائج : يعاني المرضى المصابين ب الداء البطني الغير معالجين من نقص حاد في صوديوم الدم مع نقص خفيف في كلوريد الدم (قيم P<0.001، 0.023) على التوالي، ولكن لم تتأثر تراكيز البوتاسيوم والبيكربونات والفجوة الأنيونية.

خاتمة:

لا يحدث عادة اختلال التوازن الحمضي القاعدي واضطراب الالكتروليتات عند الأطفال المصابين بمرض الداء البطني والذين يعانون من أعراض خفيفة، في حين تنخفض تراكيز كل من الصوديوم والكلورايد بسبب فقدانهم من خلال الإسهال.