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anthropometric measurements (weight, BMI, WC) in children. While; in contrary to current results; the study of Sedaghat Gohar et.al., 2021; in adults; found insignificant correlation between serum Copeptin levels with body weight, WC, TG and LDL- C when adjusted their results for age and BMI. Moreno- Navarrete et.al; 2012 found that serum Zonulin levels were positively correlated with BMI, TG and HDL- C. Tuncay et.al., 2017 found that serum Zonulin level was positively correlated with BMI and LDL- C., whereas it was negatively correlated with HDL- C in male children.

Unfortunately, no studies investigated the relation between serum Zonulin and serum Copeptin to each other in children. However, few studies discussed the relation between serum Zonulin together with serum Copeptin in adults, for example, Calgin et.al., 2019 investigated serum Zonulin and Copeptin levels in chronic hepatitis B (CHB) patients to assess the relation of viral load with intestinal permeability and systemic circulation disorders in chronic viral hepatitis patients. They found negative correlation between serum Zonulin and Copeptin levels with HBV- DNA load revealing that the biomolecules of Zonulin and Copeptin may be used for treatment monitoring in these patients.

Conclusion:

There were significant negative correlation between serum Zonulin and Copeptin among the Egyptian children. Serum Zonulin and Copeptin had significant reverse correlations with blood pressure and anthropometric measurements, and insignificant correlations with lipid profile.

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Statistical Analysis:

Collected data were complied, coded; verified and analysis was performed using the computer program SPSS (Statistical package for social science) version 16. Normality of data was tested using the Kolmogorov- Smirnov test. Most of the variables; such as the data of BMI (WC) and laboratory investigations; particularly Zonulin and Copeptin; were not normally distributed. So, the data was analyzed using non parametric tests.

Descriptive statistics (mean± standard deviation) were calculated for the anthropometric parameters and laboratory investigations. In order to find out whether there are group differences, Mann- Whitney test was carried out to compare between 2 groups for the parametric data (quantitative). Spearman´s correlation was used to assess the association between either Zonulin or Copeptin with all the studied variables. Standards of probability were set to P< 0.01; which considered highly significant; and P< 0.05; which considered statistically significant; in all analyses.

Results:

The present study included 111 children: 45 males (mean age 8.74± 1.60 years) and 66 females (mean age 8.78± 1.73 years). Table (1) showed insignificant sex differences in all the studied variables including: age, clinical, anthropometric and laboratory investigated variables. Consequently statistical analyses were done without sex differentiation.

Spearman's correlation analysis between Zonulin and Copeptin in one side and the studied clinical, anthropometric and laboratory parameters on the other side among total sample table (2), revealed that serum Zonulin had significant negative correlations, while Copeptin had significant positive correlations with age (r= 0.384, p= 0.000), DBP (r= 0.037, p= 0.011), Weight (r= 0.374, p= 0.000), Height (p= 0.002, p= 0.000), BMI (r= 0.378, p= 0.000), and WC (r= 0.353, p= 0.000). Table (3) also revealed highly significant negative correlation between both Zonulin and Copeptin (r= 0.842, P= 0.0001).

Table (1) Sex differences in investigated clinical, anthropometric and laboratory parameters among total sample (Mann Whitney test)

Parameter		Male (n= 45)		Female (n= 66)		(z)	p
		Mean	SD	Mean	SD		
Age (Years)		8.74	1.60	8.78	1.73	−0.226	0.821
Blood Pressure	SBP (mm Hg)	102.15	18.88	103.11	11.36	−0.387	0.699
	DBP (mm Hg)	63.44	12.10	63.56	7.68	−0.974	0.330
Anthropometry	Weight (Kg)	46.30	18.36	45.96	20.57	−0.003	0.998
	Height (Cm)	133.91	14.48	133.14	18.05	−0.051	0.959
	BMI (kg/m2)	24.70	6.36	24.40	7.27	−0.063	0.950
	WC (Cm)	79.24	16.66	78.83	18.66	−0.123	0.902
Lipid Profile	Chol (mg/dl)	165.82	36.48	166.62	26.05	−0.828	0.408
	TG (mg/dl)	88.51	29.11	86.00	27.40	−1.320	0.187
	HDL (mg/dl)	50.27	25.11	48.58	13.69	−0.721	0.471
	LDL (mg/dl)	66.84	10.47	66.86	8.24	−0.172	0.864
	Zonulin (ng/ml)	0.96	1.07	1.22	1.63	−0.274	0.784
	Copeptin (pmol/l)	198.67	109.92	203.12	110.41	−0.172	0.863

*P value> 0.05, insignificant difference.

Table (2) Spearman's Correlation between Zonulin and Copeptin with clinical, anthropometric and laboratory parameters among total sample

Parameter		Zonulin (ng/mL)		Copeptin (pmol/l)	
		r	P- Value	r	P- Value
Age (Years)		−0.346	0.000**	0.384	0.000**
Blood Pressure	SBP (mm Hg)	−0.151	0.119	0.167	0.086
	DBP (mm Hg)	−0.201	0.037*	0.245	0.011*
Anthropometry	Weight (Kg)	−0.335	0.000**	0.374	0.000**
	Height (Cm)	−0.289	0.002*	0.334	0.000**
	BMI (kg/m^2)	−0.347	0.000**	0.379	0.000**
	WC (Cm)	−0.333	0.000**	0.353	0.000**
Laboratory	Chol (mg/dl)	0.013	0.891	0.093	0.341
	TG (mg/dl)	−0.023	0.815	0.058	0.551
	HDL (mg/dl)	−0.154	0.111	−0.095	0.330
	LDL (mg/dl)	0.069	0.480	−0.073	0.457
	Zonulin (ng/ml)			−0.842	0.000**

*P<0.05, significant, **P<0.001, highly significant.

Discussion:

The present study revealed insignificant sex differences in all the studied variables: age, clinical, anthropometric and laboratory parameters including Zonulin and Copeptin. Consequently statistical analyses were done without sex differentiation.

In agreement with the current results (Kim et.al., 2018) and (Kozioł-Kozakowska et.al., 2020) found insignificant sex difference in the anthropometric parameters and obesity- related biomarkers among obese children and adolescents. (Reddy et.al., 2020) found insignificant sex difference in the lipid profile among overweight and obese children. Insignificant sex difference in the serum Zonulin level was reported previously by (Kim et.al., 2018); among obese children and adolescents with obesity- related biomarkers; (Ohlsson et.al., 2017) and (Lusikelelwe et.al., 2020); among obese adults. While the insignificant sex difference in the level of serum Copeptin comes in agreement with that concluded in previous studies conducted on both children and adolescents (Juliane et.al., 2016); (Yin et.al., 2020), and adults (Enhörning et.al., 2013); (Vintilă et.al., 2016).

In contrary to current results (Da Silva et.al., 2020) found obese adolescent males had significant higher body weight than females. (Asif et.al., 2021) reported that body height, weight and (WC) were higher in boys than girls with mean age of 8.87 years. (Song et.al., 2020) found significant sex difference in the lipid profile (triglycerides, cholesterol, LDL and HDL) where obese adolescent's females had higher values than males; it may be due to the difference in age group and effect of puberty.

The current results revealed that; among total sample; serum Zonulin had significant negative correlations, while Copeptin had significant positive correlations with age, DBP, and the studied anthropometric parameters (WT, HT, BMI, and WC). Concerning the laboratory investigations in the current study, there were insignificant correlations between zonulin and Copeptin on one side and any of the studied laboratory investigations.

Similarly (Yin et.al., 2020) found that serum Copeptin were significantly positively correlated with blood pressure and most of the

Introduction:

Zonulin is a human physiological protein structure which regulates the permeability of intestine. Circulating zonulin levels are considered to be a useful marker of intestinal permeability and higher circulating Zonulin levels were reported in obesity and its related metabolic disorders (Liudmyla et.al., 2021).

Arginine vasopressin (AVP) is a neuro- hormone released from the pituitary gland. It plays a role in adrenocorticotropic hormone (ACTH) secretion and lipid metabolism (Ding and Magkos., 2019). The measurement of the C- terminal fragment of AVP precursor (Copeptin) was used to estimate the AVP secretion. Copeptin is secreted in equimolar amounts with AVP. Therefore, it reflects its release and can serve as a reliable surrogate marker for circulating levels of AVP (Hanna et.al., 2021).

Copeptin, as a biomarker released into the circulation, could be a potentially promising biomarker in diagnosis of various diseases and prediction of functional outcomes. Since it is not specific to a certain disease, Copeptin could be used as an adjunct with more specific biomarkers where it may increase diagnostic accuracy and aid clinicians in making better diagnostic judgments (Raef et.al., 2020).

Aims:

This study aimed to assess the relation between plasma Zonulin and Copeptin with each other, and their relations with the metabolic disturbances risk factors in Egyptian children.

Subjects And Methods

Subjects:

This case- control cross study, included 111 children (45 males and 66 females); with age ranged between 6 up to 10 years to avoid the effect of puberty (prepubertal). It was conducted in "Management of Visceral Obesity and Growth Disturbances clinic", in "Medical Excellence Research Centre MERC", National Research Centre (NRC), Giza, Egypt, from Jenuary to Augast 2018.

Ethical Consent:

Ethical approval was obtained from both the Ethics Committee of "Faculty of Postgraduate Childhood Studies" and that of the "National Research Centre" (Approval No. 17932). Informed written consents were obtained from one of the parents after explanation of the aim of the study and its possible benefits for identifying the impact of obesity on health. This was confirmed orally and by the personally dated signature from one of the parents.

Methedology:

Each child was subjected to the following:

1. Full History Taking: Including:
   a. Personal History: Name, age (date of birth), sex and address.
   b. Present History: Onset (acute or gradual), course (i. e. the progress) and duration of gaining weight, previous diet regimens, medical attention and medications received.
   c. Past History: Birth weight, neonatal complications, type of feeding (breast feeding, artificial or mixed), and weaning age.
   d. Family History: Family history of (positive consanguinity, obesity, type 2 diabetes, cardiac disease& hypertension).
2. General Clinical Examination: Including cardiac, chest and abdominal examination; to exclude presence of any chronic or genetic disorders; with special emphasis on endocrinal diseases; that would affect the normal growth of the children.
3. Blood Pressure Measurement: Both systolic and diastolic blood pressures were measured in the sitting position using a standardized mercury sphygmomanometer with an age appropriate blood pressure cuff that cover at least two thirds of the left upper arm length and did not encroach on the antecubital space. Three successive blood pressure readings were taken, and if the error was acceptable the mean was recorded (Lee et.al., 2020).
4. Anthropometric Assessment: Anthropometric measurements; body weight, height and waist circumference; were performed using standardized equipment's following the recommendations of the International Biological Program (Hiernaux and Tanner, 1969).

   Weight was measured using a digital SECA scale balance (Model 707 standing scale). Children were weighed wearing light clothes; with no shoes; while standing on the scale with their weight equally placed on both feet. Weight was measured and recorded to the nearest 0.1 kg. Height was measured using a wall mounted Holtain Stadiometer. Each child was asked to remove his clothes, except for light underwear and stand with their feet fairly close together then asked to breathe normally. Height was measured three times and the average was recorded to the nearest 0.1 cm. Waist circumference (WC) was measured at a level midway between the lower rib margin and iliac crest using simple non- stretchable plastic measuring tape all around the body in horizontal position. The reading of the measuring tape was taken at the end of normal expiration to prevent subjects from contracting their abdominal muscles or from holding their breath. The measuring tape was held firmly, ensuring its horizontal position but loosened enough to allow the observer to place one finger between the tape and the subject's body. The readings were approximated to the nearest 0.1 cm.

   BMI was calculated using the formula: BMI= Weight (Kg)/ [Height (m)] (Krebs et.al., 2007).
5. Laboratory Investigations: A 5 ml sample of venous blood was obtained from each child between 9:00 am and 11:00 am after 12 hours of fasting. After clotting, the blood samples were centrifuged and the serum was separated and kept at −80°C for batch assessment. Professional staff performed the venipuncture for assessment of lipid profile, Zonulin and Copeptin. Serum Lipid profile was assessed using the Beckman Coulter/ Olympus AU480 Random Access Chemistry Analyzer.

   Serum Zonulin and Copeptin were measured by enzyme linked immunosorbent assay kit (ELISA) based on the principle of

Assessment of Plasma Zonulin and Copeptin Levels in Childhood

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Summary

Background: Obesity is a critical problem among children and adolescents and we in need to discover novel biomarkers for obesity and its metabolic disorders.

Objective: To assess the relation between plasma Zonulin and Copeptin and their relations with the metabolic disturbances in Egyptian children.

Methodology: Case- control study conducted in the National Research Centre (NRC); included 111 children; 45 males (8.74± 1.60 years) and 66 females (8.78± 1.73 years). History taking, clinical examination, blood pressure measurement, body weight, height, BMI and waist circumference parameters lipid profile, Zonulin and Copeptin levels were measured.

Results: Zonulin had significant negative correlations (r= 0.346, p= 0.000), Copeptin had significant positive correlations with age (r= 0.384, p= 0.000), diastolic blood pressure (r= 0.037, p= 0.011), Weight (r= 0.374, p= 0.000), Height (p= 0.002, p= 0.000), BMI (r= 0.378, p= 0.000), and WC (r= 0.353, p= 0.000). Insignificant correlations between Zonulin and Copeptin with lipid profile and significant negative correlation between Zonulin and Copeptin (r= 0.384, P= 0.0001).

Conclusion: Significant negative relations between serum Zonulin and Copeptin, and significant reverse relations with blood pressure and anthropometric measurements.

Keywords: Zonulin, Copeptin, blood pressure, anthropometry, lipid profile, Childhood.

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تقييم مستويات زونولين البلازما والكوبيتين فى مرحلة الطفولة

الخلفية: السمنة هى مشكلة حرجة بين الأطفال والمراهقين ونحن فى حاجة إلى اكتشاف مؤشرات حيوية جديدة للسمنة واضطراباتها الأيضية.

الهدف: تقييم العلاقة بين البلازما زونولين وكوبيتين وعلاقتهما بالاضطرابات الأيضية لدى الأطفال المصريين.

المنهجية: دراسة حالة ضابطة أجريت فى المركز القومى للبحوث (NRC)؛ شملت ١١١ طفلا؛ ٤٥ من الذكور (٨,٧٤± ١,٦٠ سنة) و٦٦ من الإناث (٨,٧٨ ±١,٧٣ سنة). تم قياس التاريخ المرضى، والفحص السريرى، وقياس ضغط الدم، ووزن الجسم، والطول، ومؤشر كتلة الجسم، ومعلمات محيط الخصر، ومستويات الزونولين وكوبيتين.

النتائج: كان للازونيولين ارتباطات سلبية معنوية (r= 0.346, p= 0.000)، كان للكوبيتين ارتباطات إيجابية كبيرة مع العمر (r= 0.384, p= 0.000)، وضغط الدم الانبساطى (r= 0.037, p= 0.011)، والوزن (r= 0.374, p= 0.000)، والطول (p= 0.002, p= 0.000)، ومؤشر كتلة الجسم (r= 0.378, p= 0.000)، و WC (r= 0.353, p= 0.000). ارتباطات ضئيلة بين الزونيولين والكوبيتين مع ملف تعريف الدهون وارتباط سلبى كبير بين الزونيولين والكوبيتين (r= 0.384, P= 0.0001).

الاستنتاج: علاقات سلبية كبيرة بين الزونيولين والكوبيتين، وعلاقات عكسية كبيرة مع ضغط الدم والقياسات الأنثروبومترية.

الكلمات المفتاحية: الزونيولين، الكوبيتين، ضغط الدم، القياسات الانثروبومتريه، الدهون، الاطفال.

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