Article presented at 2nd International Nineveh Conference on Medical Sciences (2nd INCMS 2023)

Short Communication

Measurement of Apelin as a Biomarker and Its Relationship to Cystatin C, Copeptin, and Glomerular Filtration Rate in Serum of Patients with Acute Kidney Injury in Mosul City, Iraq

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Abstract

Background: There is a need for early and accurate diagnosis of acute kidney injury, as high levels of cystatin C, copeptin, and low apelin levels in serum can provide an early diagnosis of acute kidney injury in patients. **Objectives:** To measure the level of apelin as a biomarker in the blood serum of both control and acute kidney injury groups, and to find its relationship with each of cystatin C, copeptin, and estimated glomerular filtration rate of both groups. **Materials and Methods:** This study was conducted on 232 blood samples collected from males and females, 108 from control subjects and 124 from patients with acute kidney injury, aged between 35 and 56 years. **Results:** Serum apelin (pg/mL) levels decreased in the blood serum of the acute kidney injury patients group, increased both cystatin C and copeptin, and decreased estimated glomerular filtration rate compared to the control group. **Conclusion:** The results showed that serum apelin levels decreased in the blood serum of the acute kidney injury group, corresponding to an increase in the level of both cystatin C and copeptin levels and a decrease in the estimated glomerular filtration rate compared to the control group, which indicates the possibility that apelin is a strong predictive biomarker for acute kidney injury, and it can play a good preventive and therapeutic role in the future.

Keywords: Acute kidney injury, eGFR, serum apelin, serum copeptin, serum cystatin C

Introduction

Acute kidney injury (AKI) is a clinical syndrome that appears precisely in the rapid or sudden deterioration in kidney function, resulting in an imbalance of electrolytes, fluid in the body, and its volume, as well as the abnormal retention of nitrogenous wastes in the body.[1]

In addition to keeping, AKI, also called acute kidney failure, occurs when the kidneys suddenly stop working, which can happen within a few hours or days. The kidney loses its ability to rid the body of waste, which leads to its accumulation in the body, and this in turn leads to the kidney losing its ability to maintain the correct balance of fluids in the body. Acute kidney failure is not present permanently in the event of immediate treatment, and if the affected person does not have serious health problems, the kidneys can return to work normally.[1]

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Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_1257_23

For this reason, to facilitate the early diagnosis of acute kidney failure, we must develop a more accurate and deeper explanation of acute kidney failure, as we must detect a more sensitive and capable marker than creatinine for early detection of acute kidney failure.[2]

The apelin gene (also known as APJ) was identified and cloned in 1993. This gene was found to be encoded by the apelin receptor, which is a novel G protein-coupled receptor (GPCR). Whereas, by cleavage of the C-terminal precursors of the 77-amino-acid, pre-pro-apelin precursor represents the peptide fragments of varying lengths that rotate *in*

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Submission: 22-Aug-2023 **Accepted:** 15-Sep-2024 **Published:** 21-Nov-2024

How to cite this article: Majeed AS, Mustafa LA. Measurement of apelin as a biomarker and its relationship to cystatin C, copeptin, and glomerular filtration rate in serum of patients with acute kidney injury in Mosul City, Iraq. Med J Babylon 2024;21:S308-12.

vivo, apelin-36, apelin-17, and apelin-13 which represent the main Asian forms.[3] Apelin plays a significant role in the cardiovascular system and human plasma and is the pyroglutamic form of apelin-13.[3] In the apelin system for kidney disease, apelin protects a wide range of diseases due to its anti-inflammatory and anti-fibrotic role. It may also have important roles in some kidney diseases. In all regions of the renal cortex and medulla, APJ mRNA was found.[4]

The presence of apelin receptor protein was also confirmed in these regions, as it was detected in the glomerular arteries and, to a lesser extent along the nephron. Studies have indicated that apelin can overcome vasoconstriction by angiotensin II. In the collecting ducts, apelin counteracted the antidiuretic effect of vasopressin, where it was found that the RAS can synthesize renin-angiotensin within the kidney as a molecular target for protective action. ELABELA (EIA), a new endogenous ligand for the apelin receptor, has been identified.[5]

Copeptin is a glycopeptide consisting of 39 amino acids, with a structure rich in lysine.[6] It is molecularly secreted in equal amounts with arginine vasopressin (AVP) from the pituitary gland and is processed during axonal transport. AVP is a highly unstable molecule whose quantification is fraught with major problems, copeptin is a stable molecule, allowing us to easily measure it as a large amount.[7] For this reason, we use the currently widespread copeptin as an alternative to AVP in various pathological conditions, including kidney disease, which is associated with many kidney diseases with relevant clinical outcomes and potential different effects of treatment.[6] Copeptin has a longer and more stable half-life, which is 26 min in front of 12 min of AVP, and this fact has stimulated research regarding copeptin as a measure of AVP in different clinical conditions. High levels of copeptin serve as a predictive marker for unfavorable outcomes in sepsis, shock, and kidney disease.[8] Studies have indicated that copeptin was increased in AKI.[7]

Cystatin C is one of the most emerging biomarkers for the diagnosis of (AKI) in patients.[9] It is a low molecular weight protein, and it is synthesized in all cells of the body and acts as an inhibitor of the protease.[9] It is released into the bloodstream at a steady rate and is excreted exclusively through the kidneys, where it is filtered through the glomerular conduction membrane and reabsorbed by the tubular epithelium, where it is almost completely degraded and does not get refiltered under normal conditions.[9] Its concentration is significantly related to improved kidney function. Where the concentration of cystatin C is considered more sensitive, especially than the concentration of creatinine, studies have indicated that age, gender, smoking status, high concentration of C-reactive protein, and other pathological conditions may affect the concentration of cystatin C in blood. Studies have indicated that all patients with AKI have elevated serum cystatin C.[10]

Estimated glomerular filtration rate (eGFR) is usually associated with decreased kidney blood flow. Inflammation is an important additional component of AKI, resulting in extended infection. Since injury to kidney cells can be fatal, sublethal injury represents an important component of acute kidney impairment, as it is likely to significantly affect glomerular filtration rate and kidney blood flow. Also, albuminuria and estimated glomerular filtration rate were independently associated with AKI, as human serum albumin level decreases in chronic and acute illnesses, which provides accurate information on the early diagnosis of illnesses.[11] Where the decrease in the glomerular filtration rate (eGFR) is considered the defining characteristic of (AKI), as it works to increase the levels of both blood urea nitrogen (BUN) and Creatinine clinically, it was found that the ability to measure the glomerular filtration rate very quickly and with high accuracy at an early stage of acute kidney failure will quickly determine the severity of the injury, which paves the way for drug or early kidney treatment or both. The decrease in (eGFR) may help in understanding the mechanism of kidney disease and provide new strategies for its prevention.[12] The study aimed to measure the level of apelin as a biomarker in the blood serum of both control and AKI groups and find its relationship with each of cystatin C, copeptin, and estimated glomerular filtration rate of both groups.

Material and Methods

Subjects

During a period from May 2022 to February 2023, subjects were recruited from Ibn Sina, Al-Salam and Al-Jumhuris teaching hospitals, and the Central Blood Bank in Mosul City, Iraq.

Patients

During the study, 232 blood samples were collected from males and females, 108 from the control group and 124 from patients with AKI, aged between ≤35 and ≥56 years). A total of 10 mL of blood were drawn from the subjects. Serum samples were separated and then kept in clean and tightly covered tubes at a temperature of −20°C until use.

Exclusion criteria

The collected samples were free from decomposition and turbidity to avoid any interference with the results, as well as free from viral hepatitis.

Methods

Serum human apelin is determined by using an ELISA kit assayed according to the manufacturer's procedure (Bioassay laboratory technology, Cat. No. EH 2174, China).[13] Serum human cystatin C (CST:3) is determined by using an ELISA kit assayed according to the manufacturer's procedure (Bioassay laboratory technology,

Cat. No. EH 0110, China).[13] Serum human copeptin (CPP) is determined by using an ELISA kit assayed according to the manufacturer's procedure (Bioassay laboratory technology, Cat. No. EH 2880, China).[13]

The glomerular filtration rate was calculated using the Cockcroft-Gault formula.[13] The study was conducted at the Department of Life Sciences at the University of Mosul, Iraq, in partnership with Al-Ghufran Laboratory in Mosul.

Statistical analysis

In the study, the SPSS statistical analysis system was used to analyze the results. An independent *t*-test was performed to analyze the results of the study involving two variables, while a completely randomized design (CRD) was used to analyze the variance of traits that included more than two variables, using one-way analysis of variance, as well as extracting the correlation coefficient for some of the school characteristics, and the correlation factor, as well as extracting the means and standard error. Duncan's multiple range test was applied to find the significant differences between means, with a significance reduction score ($P \leq 0.05$).[14]

Ethical approval

The study was conducted by the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and written approval before the sample was taken. The study protocol information and consent form were reviewed and approved by a local ethics committee according to document number 14459 (including the number and the date on April 26, 2022.

Results

The results shown in Table 1 indicate that there is a significant decrease in the level of apelin at the level of probability ($P \leq 0.05$) in patients with AKI (148.600 ± 11.67 pg/mL) compared to the control subject (1777.99 ± 88.14 pg/mL). The results in Table 1 also show that there is a significant increase in the level of cystatin hormone concentration at the level of probability ($P \leq 0.05$) in the blood serum of patients with low apelin hormone in AKI (13.70 ± 0.45 ng/mL).

A significant increase was shown in the level of copeptin (99.21 ± 4.45 pg/mL) at the probability level ($P \leq 0.05$). Additionally, a significant decrease was found at the probability level ($P \leq 0.05$) in the level of glomerular filtration rate (eGFR) (48.22 ± 3.97 mL/minute) in patients.

Correlation between apelin and biochemical parameters measured in the serum of patients with AKI:

The relation of apelin with the biochemical variables measured in the blood serum of patients with AKI, as shown in Table 2, was studied by finding the linear correlation coefficient (*r*).

A direct significant relation at the level of probability ($P \leq 0.0$l), shown in Table 2, between the concentration of apelin and the level of glomerular filtration rate (eGFR), indicates that the correlation between them is positive and significant.

In contrast, the negative significant correlation between the concentration of apelin and the level of concentration of both cystatin C and copeptin in patients with AKI indicates that the concentration of apelin decreases in patients.

The effect of sex on the level of biochemical parameters measured in the blood of patients with AKI.

The effect of sex factor was studied on the level of all biochemical parameters that were measured in the serum of patients with AKI, as shown in Table 3 and Figure 1. There was a significant increase in the concentrations of cystatin C and copeptin in male patients compared to female patients, a significant decrease in the concentration of glomerular filtration rate (eGFR) in male patients compared to female patients and no significant differences shown between male and female groups in other biochemical parameters.

- The similar letters in one column horizontally indicate that there are no significant differences at a significant level $P \leq 0.05$
- The different letters in one column horizontally indicate the existence of significant difference at a significant level $P \leq 0.05$

Table 1: Level of biochemical parameters measured in the blood serum of patients with acute kidney injury (AKI) compared to control subjects

Biochemical parameters	Control subjects $N = 108$		Patients group $N = 124$		*P*-value
	Mean	±SE	Mean	±SE	
Apelin (pg/mL)	1777.99*	88.14	148.600	11.67	$P \leq 0.05$
Cystatin C (ng/mL)	9.94*	0.29	13.70	0.45	$P \leq 0.05$
Copeptin (pg/mL)	40.43*	1.21	99.21	4.45	$P \leq 0.05$
eGFR (mL/min)	155.55*	2.85	48.22	3.97	$P \leq 0.05$

*The difference is significant horizontally at the level of significance $P \leq 0.05$

Table 2: Correlation between apelin and biochemical parameters measured in the serum of patients with acute kidney injury

Biochemical parameters	Patients group $n = 124$	
	r-value	*P*-value
Cystatin C (ng/mL)	−0.764**	0.000
Copeptin (pg/mL)	−0.371**	0.000
eGFR (mL/min)	0.777**	0.000

**Indicates that there is a significant difference in the probability value $P \leq 0.01$

Table 3: The effect of sex on the level of biochemical parameters measured in the blood of patients with acute kidney injury

Biochemical parameters	Males $N = 62$		Females $N = 62$	
	Mean	±SE	Mean	±SE
Apelin (pg/mL)	98.82[a]	5.25	157.50[a]	13.08
Cystatin (ng/mL)	15.27[c]	0.52	11.87[b]	0.66
Copeptin (ng/mL)	119.65[c]	6.63	80.00[b]	4.77
eGFR (mL/min)	21.74[a]	1.25	35.88[b]	3.36

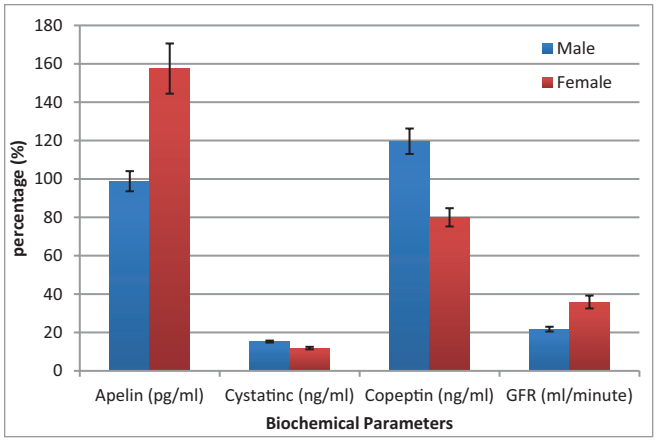

Figure 1: Effect of sex on level of (apelin, cystatin C, copeptin, eGFR) in serum of acute kidney injury (AKI) patients

Discussion

The reason for the decrease in the level of apelin is attributed to increases in plasma osmolality that were accompanied by a simultaneous increase in plasma AVP levels and a decrease in plasma apelin levels. Thus, there is an abnormal apelin/AVP balance in AKI patients, which leads to higher levels of water reabsorption by the kidney tubules. Decreased water diuresis leads to an elevation of AVP and a decrease in apelin levels.[15] The reason for the significant increase in the level of cystatin C at the level of probability ($P \leq 0.05$) is that cystatin C is an endogenous biomarker of kidney function that is produced by all nucleated cells at a near-constant rate, regardless of muscle mass, and is cleared from circulation through glomerular filtration without reabsorption or secretion.

Cystatin C is evaluated as an inflammatory biomarker, making it accurate for kidney injury. This result was compatible with several authors worldwide.[16,17]

The reason for the significant increase in the level of copeptin at the level of probability ($P \leq 0.05$) can be explained by the two proposed mechanisms. The first mechanism shows that when copeptin is cleared by kidney excretion, levels of copeptin tend to increase with decreased kidney function, and the second mechanism shows that, in patients with decreased kidney function, more copeptin is released because the AVP system is activated due to the poor ability of urine concentration to maintain water balance as a result of the disruption of the apelin/AVP systems.[15] Since copeptin is a derivative of the cleavage of the precursor of AVP, it produces equal proportions in the hypothalamus, where copeptin is a stable part and has a longer half-life than AVP, which is considered a byproduct. It is stable both *in vivo* and *in vitro* and has a short half-life of 5–20 min. Therefore, copeptin is measured instead of AVP and serves as a predictive clinical marker for AKI. This result was consistent with the findings of Afsar *et al.*[7]

The reason for the significant decrease in the probability ($P \leq 0.05$) in the level of eGFR is due to the increased concentration of copeptin being associated with AKI, as copeptin is negatively associated with the estimated glomerular filtration rate (eGFR). This was consistent with several authors.[7,17]

The reason is that the association between apelin and eGFR is positive and significant at the probability level ($P \leq 0.01$). The concentration of apelin decreases in AKI and is associated with a decrease in the glomerular filtration rate (eGFR).[7]

The binding of apelin to both cystatin C and copeptin was negative and significant and corresponds to an increase in the concentration of both cystatin C[17] and copeptin[7,15] corresponding to a decrease in the level of apelin.

Conclusion

The results showed the serum apelin decreased in the blood serum of the AKI group, corresponding to an increase in the level of both cystatin C, copeptin, and a decrease in the estimated glomerular filtration rate compared to the control group, which indicates the possibility that apelin is a strong predictive biomarker for AKI, and it can play a good preventive and therapeutic role in the future.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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