Title: Clinical Value of Plasma Humanin in Acute Kidney Injury

Date: Augest 1,2022

The research plan

1. Clinical Value of Plasma Humanin in Acute Kidney Injury

1.1 Study design

This study is a single-center cross-sectional study, observed and analyzed patients with acute and chronic kidney disease from August 2022 to June 2024, according to the following criteria: adult patients aged 18 years, both male and female; AKI patients: acute kidney injury patients meeting the definition of KDIGO guidelines; control group: people from healthy examination, with normal blood routine physical examination, urine routine, liver function, renal function, glycosylated hemoglobin; patients had complete baseline medical records, including renal and kidney function, urinary protein level, blood creatinine value; agreed to join the group and signed informed consent;

Population was excluded by the following criteria: patient age <18 years; patient baseline data was missing.

1.2 Relevant Definitions

According to the 2012 Global prognosis (KDIGO) criteria, acute kidney injury (Acute Kidney Injury, AKI) was defined as: blood creatinine level increased 0.3 mg/dl (26.5 μ mol / L) or more than 1.5 times or above the base value, and clearly or inferred to occur within 7d; or continuous 6h urine volume <0.5ml kg- ¹ h- ¹ ;

1.3Data collection

Observational analysis of patients with acute kidney injury from August 2022 to June 2024 in our hospital, and collected plasma from patients meeting the inclusion criteria and divided into AKI group and control group

Clinical baseline data of patients meeting the inclusion criteria, including gender, age, blood pressure, underlying diseases (hypertension, diabetes, hyperuricemia), serum creatinine, eGFR, serum albumin, hemoglobin concentration, glycated hemoglobin, cholesterol, triglycerides, high-density lipoprotein, low density lipoprotein, uric acid, blood phosphorus, blood calcium, potassium, serum iron, 24h urinary protein / urinary albumin, urinary protein / urinary albumin creatinine ratio.

2.plasma Humanin concentration detection

2.1 Samples to be taken

Patients who met the inclusion criteria were retained for 4ml of blood / sterile urinary urine with an EDTA tube during hospitalization Within 2h, after centrifugation (3000 r/min, 10min), take the supernatant at 300-600 μ in the-80℃ refrigerator.

2.2 Experimental reagent consumables and instruments

Human humanin peptide (MT-RNR 2) ELISA kit, centrifuge, EP tube, different specifications of enzyme-free gun head, different specifications of pipete gun and exhaust gun, plasma water, microplate reader, 4℃ refrigerator, -20℃ refrigerator, -80℃ refrigerator, 37℃ incubator, etc

2.3 Experimental method

（1）Reagent reheating: Transfer all reagents to room temperature (18-25C) to equilibrate for at least 30 minutes

（2）Reagent equipped with:

① Standard: Remove a standard from the kit at 6000-10000rpm for 30 seconds. Dissolve with 1ml sample dilution, pump the gun at the bottom of the cold storage tube for 5 times to dissolve the standard S7; reserve 71.5ml centrifuge tubes with different concentrations (use the following concentrations: 1800,900,450,225,112.5,56.25,28,0 pg / ml);

② Washing liquid working liquid: the concentrated washing liquid should be diluted by 1:25 times with de-ionized water, and qualified before use;

③ Biotin-labeled antibody working solution: biotin-labeled antibody solution is diluted 1:100 times and consummated within 10 minutes before use;

④ Horseradish peroxidase labeled avidin working solution: horseradish peroxidase labeled avidin by 1; 100 times with horseradish peroxidase labeled avidin dilution, within 10 minutes before use;

⑤ Sample dilution: Samples are diluted 1:5 times for testing;

（3） Add samples: set up standard holes and sample holes to be tested respectively. Add 100u1 to each well, shake the well gently, and warm 37C for 2 hours.

（4） Leave the liquid and dry without washing.

（5）100u1, covered with new plates, and incubated at 37C for 1 hour.

（6） Remove the liquid in the hole, throw it on, and wash the plate for 3 times. Soak for 2 minutes, 200u / each well and dry.

（7） Horseradish peroxidase-labeled avidin working solution was 100u1 per well, covered with new plate paste and incubated at 37° C for 1 hour.

（8） Remove the liquid in the hole, shake it dry, and wash the plate for 5 times. Soak for 2 minutes, 200ul / per well and dry.

（9） Add substrate solution 90u1,37C for 15-30 min.

（10）The reaction was terminated by adding 50u1 of the termination solution per well.

（11） The optical density (OD) of each well was measured at 450nm within 5 minutes of the reaction

（12）Calculated concentration: According to the standard concentration and the corresponding OD value, the standard curve of Humanin is analyzed by Curve Expert 1.4 software, and the concentration of Humanin to be measured in the sample is calculated by using the equation of the standard curve.

3. Statistical methods

SPSS software. Measurement data following normal distribution are described by ($x \pm s$) and t test was used for comparison between groups. Count data are represented by frequency, percentage (%), and chi-square test. The receiver working characteristic curve (ROC) was drawn to analyze the diagnostic value of Humanin for AKI. A $P < 0.05$ was considered as a statistically significant difference.