Dietary Acid Load and Glomerular Filtration Rate in Chronic Kidney Disease

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Abstract

**Background**: Kidney diseases are prevalencing rapidly. The nutritional transition has caused the diet of Iran community to increase the dietary acid load (DAL) and thus exacerbate metabolic disorders. Therefore, our goal was to determine the DAL of the diet in patients with Chronic Kidney Disease (CKD).

**Methods**: In this cross-sectional study, the study population was composed of 90 patients with renal insufficiency. DAL was included of Potential Renal Acid Load (PRAL), Net EndogeneusAcid Production (NEAP) and Net Acid Excretion (NAE) that were extracted using data of food frequency questionnaire and their correlation with anthropometric and biochemical indices such as Glomerular Filtration Rate (GFR) and creatinine were analyzed by SPSS v.26 software with a significance level of < 0.05.

**Results**: Mean of dietary acid load of participants were 17.15±9.85, -8.7±0.35 and 59.04±10.9 mEq/day for PRAL, NEAP and NAE respectively. Daily intake of energy (P<0.001) and protein percent of energy (P<0.01) in third tertile (T3) of PRAL were significant higher than first tertile (T1). Mean of age (P<0.05) and blood creatinine concentration (P<0.01) were significant higher in T1 than T1 of NEAP index and GFR was significant low (P<0.05). Daily energy intake (p<0.05), blood calcium concentration (p<0.05) and GFR (p<0.05) were significantly more in higher tertiles of NAE index. Based on Crude General linear model, the higher tertiles of PRAL compared to first tertile had significant lower GFR (P<0.05). In adjustment model, T3 group had more not significant GFR than T1 group. Only in model II adjustment, T2 group of NAE compared to T1, had higher GFR. Mean difference of GFR did not significant across tertiles of NEAP index. In case of Creatinine, based on model I adjustment, T3 group of NAE had more creatinine concentration than T1 group (P<0.01). T3 group of NEAP than T1 group had significant lower creatinine in crude and model I adjustment (P<0.01).

**Discussion**: Dietary Acid Load was associated with kidney function in CKD patients. In order to obtain logical results and to understand the cause-and-effect relationships, long-term studies with larger populations and consideration of blood factors such as blood bicarbonate are recommended.

Background

Todays, CKD is considered as international public health problem (1, 2). In addition to racial and genetic susceptibility, different risk factors such as hypertension, diabetes and lifestyle can predict CKD incidence(3). According to various studies, this disease affects between 8% and more than 16% of adults (3, 4). This no communicable disease can lead to cardiovascular morbidity, pulmonary hypertension, infection, periodontal disease, depression, dialysis, renal replacement and evently mortality (5-10). It has been shown not only in advanced stages, but in early stages of renal dysfunction, morbidity and even mortality are high (11) and taking care of this disease involves a lot of economic costs, So, preventing its modifiable risk factors such as diet can be effective in reducing costs and increasing life expectancy(12, 13). Proteins from meat, fish, eggs, cereals, and dairy products(14) are major source of nonvolatile acid and metabolized to sulfates and other organic acids while fruit and vegetables have natural potassium salts that induce alkaline condition in body(15). High DAL increases ammonium concentration and hydrogen ions excretion in kidney tubules; so these pathophysiological mechanisms, enhances activity of the renin-angiotensin system and aldosterone, causing tubular toxicity, destruction of renal nephrons and decreased renal function in long term period(11, 13). DAL
is measured by PRAL and the NEAP indexes which are based on dietary intakes of protein, calcium, magnesium, potassium and phosphorous (16). A part of the acid excreted by the kidneys can be considered equivalent to PRAL and a part as organic acid (OA), dependent on the body surface and permanently, is excreted from the kidneys (17). Considering the inappropriate diet pattern in Iranian families, such as low consumption of fruits and vegetables and high consumption of cereal and meat based foods, it is predictable that the Iranian diet will increase the acid load and thus exacerbate metabolic disorders (18). This type of diet, which is mostly the result of nutritional transition, can be associated with an increase in the acidic load of the diet and thus the aggravation of metabolic disorders; This condition is more dangerous in kidney patients, because these patients are restricted in consuming alkaline foods. In addition, because few studies have been done on the relationship between dietary acidity and kidney function (1, 19), we estimated PRAL and NEAP and evaluated their association with sociodemographic and biochemical factors in general hospital, southwest of Iran.

**Methods**

**Population and study design**

In this cross-sectional study, 90 CKD patients with GFR 20-65ml/min (20) were enrolled by easy and accessible sampling method in 2018. The protocol of this study was reviewed and approved by the Ethics Committee of Yasuj University of Medical Sciences (Ethical code:IR.YUMS.REC.1396.29). Exclusion criteria were acute or chronic inflammatory disease, malignancy or known hematological disorder and recent severe hemorrhagic episode. Patients were informed the study goals and written consent was obtained. Demographic information was obtained during interviews with them. The weight of participants was measured with light clothing by a standard scale (Seca786, Germany) with 0.1 kg accuracy. Patients’s height was also measured in Standing without shoes position by no elastistic gauge plate (Seca786, Germany) with 0.5 cm accuracy. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m²). Four groups of BMI: lean (<18.5 kg / m²), normal (18.5-24.9 kg / m²) and overweight (25-29.9 kg / m²) and obese (≥ 30 kg / m²) (7). Food intake was obtained using a semiquantitative standard food frequency questionnaire (15,19). Cronbach's alpha coefficient for our FFQ was 0.932, so considering that it is higher than the cut of point of 0.7, it can be said that the FFQ used in this study has a very good validity and reliability. Amount and frequency of consumption of food items was as daily, weekly, monthly and seasonal. It should be noted that units of use for each item were standard units; For example, how much and how many a 240cc cup for milk? For fruits such as apples, the unit used was an average of 100 grams of apples. For different types of bread, the scale used is the palm of the hand (10 x 10 cm cut). Consumption of each food item was converted in to grams of food consumed per day using the illustrated guidebook of home scales. Since in Iran, most fats are consumed as cooking oil, a separate question was included in the questionnaire; “How many kilograms of cooking oil is bought for a household? How long is it generally enough? “ Then, by knowing the number of household members, the approximate daily consumption of oil for each person can be calculated. Macronutrients and micronutrient content of each food was extracted using the Iranian food composition table. Blood samples were taken from patients after 12-14 hours of overnight fasting by an experienced nurse and biochemical tests were performed using the enzymatic colorimetric kits (bionic kit, Iran). Blood pressure is also measured by an experienced nurse using a standard mercury sphygmomanometer (DDM, Inc, Castelculier, France) after hospitalization and 15-minutes of rest in seated position.
Definitions

Dietary acid load was estimated using the Remer and Manz equation (17, 20):

\[ \text{PRAL}_{\text{mEq/day}} = 0.49 \times \text{protein} + 0.037 \times \text{phosphorus} - 0.021 \times \text{potassium} - 0.026 \times \text{magnesium} - 0.013 \times \text{calcium} \]

\[ \text{NEAP}_{\text{mEq/day}} = 54.5 + (\text{Pro:K}) - 10.2 \]

\[ \text{NAE}_{\text{mEq/day}} = \text{PRAL}_{\text{mEq/day}} + 41 \times \text{Body Surface Area (m}^2)/1.73 \text{m}^2. \]

The higher the estimated values of these variables, the higher the acidity of the diet.

Body surface area was calculated based on Mosteller formula (21). GFR was calculated from the Cockroft formula (22) or \( \text{GFR} = (140 - \text{age}) \times \text{Weight} / \text{Pcrea} \times 72 \)

Pcrea: Plasma concentration of creatinine.

Diabetes mellitus is defined as having fasting blood sugar (FBS) \( \geq 126 \text{ mg/dL} \) or taking blood sugar-lowering drugs. Hypertension was also defined as systolic blood pressure \( \geq 140 \text{ mmHg} \) or greater diastolic blood pressure \( \geq 90 \text{ mmHg} \) or taking the anti-hypertension medication (19). \( \text{BUN} > 40 \text{mg/dL} \) was considered as high BUN concentration. Albumin concentration < 2.5 g/dl was considered as marked hypoalbuminemia and mild hypoalbuminemia as 2.5-3 g/dl and normal range was 3.6-4.5 g/dl (23).

Statistical Analysis: All statistical analyses were performed using SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA). Normality of variables was assessed using the Kolmogorov–Smirnov test. Numerical variables are expressed as the mean±standard deviation and categorical ones as number(percent). Comparison across tertiles of PRAL, NEAP and NAE indexes was done using the \( \chi^2 \) test for categorical variables and One-way ANOVA and Kruskal–Wallis tests for continuous variables. The mean differences of the main dependent variables (GFR, Creatinine concentration as indicators of kidney function) were compared across the tertiles of independent variables (PRAL, NEAP and NAE indexes) using Univariate General Linear Model test in the crude model and the adjustment model (confounding variables were included the age, gender, BMI, daily energy intake, diabetes and hypertension status). \( P \)-values less than 0.05 were considered statistically significant.

Results

Mean and standard deviation of dietary acid load of participants were 17.15±9.85, -8.7±0.35 and 59.04±10.9 mEq/day for PRAL, NEAP and NAE respectively. There was a high correlation between these indicators (PRAL, NEAP: \( r=0.746, P<0.001 \)), (PRAL, NAE: \( r=0.897, P<0.001 \)), (NEAP, NAE: \( r=0.702, P<0.001 \)). The mean age and Body Mass Index (BMI) of patients was 59.93±15.12 years and 25.55±5.17 kg/m\(^2\) respectively; 52.7% of them was female. In this study, 44 (48.9%) participants had normal weight; 29 (32.2%) of them was overweight and 17 (18.9%) patients was obese. No one of them was underweight. 3.3%, 26.7% and 70% of patients had marked hypoalbuminemia, mild hypoalbuminemia and normal albuminemia respectively. Almost, all of patient had high blood creatinine concentration (Crea > 1.2 mg/dl); only one patient had normal blood creatinine concentration. 45.5% of patients had high BUN concentration. One of patients had GFR > 60 ml/min and others had lower GFR. Value of demographic, anthropometric, biochemical and blood pressure
characteristics of patients across the Tertiles of PRAL was presented in Table-1. Daily intake of energy (P<0.001) and protein percent of energy in T₃ were significant higher than T₁ (P<0.01). Other variables were not significant difference across the tertiles of PRAL index. Mean of GFR, Albumin, DBP and fat percent of energy were higher in T₁ than T₃ group. Also, age, BUN, Creatinine, SBP and protein and carbohydrate percent of daily energy were also, lower; but not significant.

Table1: characteristic of CKD patients based on tertiles of PRAL
| tertile | Total (0.08-54.64) | T1 (0.08-12.5) | T2 (12.64-18.41) | T3 (18.41-54.64) | P-value |
|---------|---------------------|----------------|-----------------|-----------------|----------|
| variable |                     |                |                 |                 |          |
| **Demographic & blood pressure** |                     |                |                 |                 |          |
| Age(y) | 59.93 ±15.1 | 56.66±15.76 | 58.9±14.5 | 64.23±14.56 | 0.138 |
| Sex(M/F) | 43/47 | 18/12 | 11/19 | 14/16 | 0.192 |
| BMI(kg/m2) | 25.55±5.17 | 25.4±4.9 | 26±4.25 | 25.3±6.3 | 0.576 |
| Diabetes(n(%)) | 27(30.3) | 7(23.3) | 11(36.7) | 9(31) | 0.529 |
| Hypertension(n(%)) | 60(67.4) | 19(62.3) | 20(66.7) | 21(72.4) | 0.754 |
| SBP(mHg) | 134±17.9 | 133±14 | 133.7±20 | 135.3±194 | 0.924 |
| DBP(mHg) | 79.2±17.25 | 78.9±15.5 | 82.1±19.7 | 76.6±16.4 | 0.544 |
| **Biochemical** |                     |                |                 |                 |          |
| Na(mg/dl) | 140.7±3.6 | 141.5±4.1 | 140.3±3.6 | 140.3±2.9 | 0.323 |
| P(mg/dl) | 4.37±1.16 | 4.4±1.05 | 4.3±1.2 | 4.37±1.16 | 0.879 |
| K(mg/dl) | 4.25±0.66 | 4.2±0.54 | 4.2±0.7 | 4.35±0.75 | 0.611 |
| Ca(mg/dl) | 8.29±1.5 | 8±1.8 | 8.2±1.6 | 8.6±1.03 | 0.302 |
| Alb(gr/dL) | 3.62±0.55 | 3.67±0.56 | 3.66±0.54 | 3.54±0.55 | 0.592 |
| BUN(mg/dl) | 41.85±20 | 40.8±12.04 | 46.2±26.5 | 38.5±18.6 | 0.207 |
| Crea(mg/dl) | 4.33±2.2 | 4.8±2.3 | 4.1±1.75 | 4.1±2.5 | 0.25 |
| GFR(ml/min) | 22.27±12.46 | 21.6±14 | 21.55±9.8 | 23.6±13.4 | 0.481 |
| **Dietary intake** |                     |                |                 |                 |          |
| Vegetable(gr/day) | 68.4±14.8 | 69.9 ±13.1 | 68.5±14.4 | 66.8±16.7 | 0.725 |
| Dairy food(gr/day) | 38.9 ±12 | 42.2±12.6 | 38±12.5 | 36.4 ±10 | 0.155 |
| Meat(gr/day) | 82.6 ±17 | 88±12.6 | 79.4 ±19 | 80.6 ±18.2 | 0.150 |
| Fruit(gr/day) | 79.3 ±17.8 | 78.7 ±14 | 80±22 | 79.3 ±17.8 | 0.319 |
| Grains(gr/day) | 38 ±7.8 | 40 ±7 | 36.2±8 | 37.7±7.8 | 0.879 |
| Others(gr/day) | 463±254 | 4601±165 | 500±320 | 431±254 | 0.283 |
| energy(kcal/day) | 1990.5±577 | 1809±527 | 1844±404 | 2317±645 | 0.001 |
| FAT. percent(%/E) | 37.5 ±9.2 | 38 ±9 | 37.7±10.3 | 36.7 ±8.5 | 0.889 |
| CHO. Percent(%/E) | 52.8±12 | 50.5 ±9 | 51.5±8 | 56.4 ±16.7 | 0.265 |
| PRO.percent(%/E) | 11.4 ±2.4 | 10.8 ±2.4 | 10.6±2.2 | 12.6 ±2 | 0.003 |

Data are showed as mean±standard deviation. The One Way ANOVA(for age, daily intake vegetable, dairy food and meats, daily intake of fruits, grains and other group, total energy, fat percent of energy, carbohydrate
percent and protein percent, blood concentrations of Albumin, calcium and potassium) , Kruskal- Wallis H test (BMI, GFR, Systolic and Diastolic Blood Pressure, Blood concentration of Calcium, Potassium, BUN, Creatinine) and Chi-square test for Sex, diabetes and Hypertension status was used.

According the Table-2, mean of age (P<0.01) and blood creatinine concentration (P<0.01) were significant higher in T3 than T1 of NEAP index and GFR was significant low (P<0.05). Other variables had no significant difference; But, T1 participants compared to T3 ones intake lower total energy, had lower GFR and protein percent of energy but had higher BUN, Creatinine; But not significant.

Table2: characteristic of CKD patients based on tertiles of NEAP
| tertile          | T1          | T2          | T3          | P-value |
|-----------------|-------------|-------------|-------------|---------|
| variable        |             |             |             |         |
| Age(y)          | 55±13       | 59.06±17.1  | 65.7±13.5*  | 0.006   |
| BMI(kg/m²)      | 24.7±4.4    | 27.2±5.7    | 24.7±5      | 0.091   |
| Sex(M/F)        | 17(13)      | 11(19)      | 15(15)      | 0.287   |
| Diabetes(n%)    | 6(20)       | 11(37.9)    | 10(33.3)    | 0.296   |
| Hypertension(n%)| 18(60)      | 22(73.3)    | 20(69)      | 0.532   |
| SBP(mHg)        | 133.2±15.2  | 132.6±20.6  | 136.1±17.5  | 0.761   |
| DBP(mHg)        | 78.8±16     | 79.1±23.8   | 79.7±9.3    | 0.932   |
| biochemical     |             |             |             |         |
| Na(mg/dl)       | 140.3±4.7   | 140.8±9.6   | 140.9±2.6   | 0.95    |
| P(mg/dl)        | 4.5±1       | 4.5±1.1     | 4.4±1.3     | 0.308   |
| K(mg/dl)        | 4.2±0.5     | 4.2±0.7     | 4.4±0.8     | 0.523   |
| Ca(mg/dl)       | 8.3±1.6     | 7.8±1.6**   | 8.7±1.3     | 0.082   |
| Alb(gr/dL)      | 3.7±0.5     | 3.6±0.5     | 3.5±0.65    | 0.489   |
| BUN(mg/dl)      | 44.3±13.3   | 44.4±28.3   | 36.9±14.6   | 0.094   |
| Crea(mg/dl)     | 5.2±2.2     | 4.1±2.3     | 3.6±1.8     | 0.008   |
| GFR(ml/min)     | 18.4±10.6   | 23.8±12.2   | 24.7±13.8   | 0.038   |
| Dietary intake  |             |             |             |         |
| Vegetable(gr/day)| 68 ±12.8    | 70.4 ±13    | 66.5 ±18    | 0.602   |
| Dairy food(gr/day)| 41 ±13     | 39 ±9.4     | 36.5 ±12.8  | 0.338   |
| Meat(gr/day)    | 84.7±16     | 86.3 ±13    | 77 ±20.2    | 0.169   |
| Fruit(gr/day)   | 77.6±15     | 83 ±16.8    | 77.2 ±21    | 0.123   |
| Grains(gr/day)  | 39.5±7.8    | 38 ±5.4     | 36.3 ±9.7   | 0.308   |
| Others(gr/day)  | 426±142     | 501 ±314    | 466 ±279.2  | 0.654   |
| energy(kcal/day)| 1864±587.3  | 1904 ±402   | 2203 ±668   | 0.067   |
| Fat percent(%/E)| 38.4±9      | 38.3 ±8.4   | 35.6 ±10    | 0.387   |
| CHO.percent(%/E)| 53.5±9      | 50.2 ±8     | 54.7 ±17    | 0.26    |
| PRO.percent(%/E)| 10.9±2.5    | 11 ±2       | 12 ±2.4     | 0.235   |

Data are showed as mean±standard deviation. The One Way ANOVA(for age, daily intake vegetable, dairy food and meats, daily intake of fruits, grains and other group, total energy, fat percent of energy, carbohydrate.
percent and protein percent, blood concentrations of Albumin, calcium and potassium) Kruskal-Wallis H test(BMI, GFR, Systolic and Diastolic Blood Pressure, Blood concentration of Calcium, Potassium, BUN, Creatinine) and Chi-square test for Sex, diabetes and Hypertension status was used.

Table 3 shows that mean of daily energy, calcium blood concentration and GFR increased significantly (P<0.05) across the tertiles of NAE index. Intake of dairy foods and vegetables, Protein and carbohydrate percent, age, BMI, SBP and GFR increased across the tertiles, although not significantly; but intake of fruit and meats, fat percent, DBP, BUN and Creatinine decreased.

**Table 3: Characteristic of CKD patients based on tertiles of NAE**

Data are showed as mean±standard deviation. The One Way ANOVA (for age, daily intake vegetable, dairy food and meats, daily intake of fruits, grains and other group, total energy, fat percent of energy, carbohydrate percent and protein percent, blood concentrations of Albumin, calcium and potassium) Kruskal-Wallis H test(BMI, GFR, Systolic and Diastolic Blood Pressure, Blood concentration of Calcium, Potassium, BUN, Creatinine) and Chi-square test for Sex, diabetes and Hypertension status was used.

According to table 4, based on crude model, mean difference of GFR in T2 and T3 of PRAL index was significant lower (P<0.01) compared to T1 group. In model II adjustment, T2 group of NAE compared to T1, had significant higher GFR (P<0.05). Mean difference of GFR did not significant across Tertiles of NEAP index (crude or adjustment models).

**Table 4: Mean Difference (M.D) of GFR across of tertiles of PRAL, NEAP and NAE in CKD patients**

| variable | tertile | T2                  | T3                  |
|----------|---------|---------------------|---------------------|
|          | Adjust model | M.D | P       | 95%CI | M.D | P   | 95%CI |
| GFR NAE  | crude®  | 4.8  | 0.237  | -3.25 | -12.9 | 7.64 | 0.092 | -1.27 | 16.5  |
|          | Model I | 1.1  | 0.837  | -9.2  | -11.3 | -16.33 | 0.431 | -57   | 24.8  |
|          | Model II | 17.9 | 0.031  | 1.65  | -34   | -43.7 | 0.304 | -128.3 | 40.8  |
| PRAL     | crude   | -12.72 | 0.003 | -20.95 | -4.49 | -14.04 | 0.002 | -22.56 | -5.5  |
|          | Model I | -24.13 | 0.132 | -55.7 | -7.5 | -4.3 | 0.596 | -20.4 | 11.8  |
|          | Model II | -11.04 | 0.337 | -33.8 | -11.8 | 30.87 | 0.394 | -41   | 102.8 |
| NEAP     | crude   | 1.09  | 0.783  | -6.79 | -8.9 | -0.46 | 0.917 | -9.23 | 8.34  |
|          | Model I | 4.8  | 0.597  | -12.4 | -21.4 | 10.6 | 0.351 | -12   | 23.2  |
|          | Model II | -6.8 | 0.619  | -30.5 | -18.3 | 30.7 | 0.136 | -9.9  | 71.2  |
| tertile          | T1          | T2          | T3          | P-value |
|-----------------|-------------|-------------|-------------|---------|
| variable        | (38.58-54.51) | (54.83-60.22) | (38.58-94.75) |         |
| Demographic & blood pressure |             |             |             |         |
| Age(y)          | 56.7±19     | 58.7±11.2   | 64.4±13.5*  | 0.124   |
| BMI(kg/m^2)     | 24±4.7      | 25.2±4.2    | 27.5±6      | 0.058   |
| Sex(M/F)        | 12(18)      | 17(13)      | 14(16)      | 0.429   |
| Diabetes=yes/no | 6(24)       | 8(21)       | 13(17)      | 0.124   |
| Hypertension=yes/no | 18(12) | 22(8)       | 20(10)      | 0.532   |
| SBP(mHg)        | 133.4±17.3  | 132.3±16.4  | 136.3±20    | 0.65    |
| DBP(mHg)        | 79.4±17.2   | 81.5±18.2   | 76.7±16.5   | 0.443   |
| Biochemical     |             |             |             |         |
| Na(mg/dl)       | 140.3±3.6   | 141.6±4     | 140.2±3     | 0.109   |
| P(mg/dl)        | 4.2±1       | 4.6±1.3     | 4.3±1.2     | 0.282   |
| K(mg/dl)        | 4.2±0.6     | 4.1±0.6**   | 4.5±0.7*    | 0.052   |
| Ca(mg/dl)       | 7.7±1.8     | 8.6±1.4*    | 8.3±1.5*    | 0.016   |
| Alb(gr/dL)      | 3.7±0.5     | 3.5±0.7     | 3.6±0.4     | 0.257   |
| BUN(mg/dl)      | 43.1±25.8   | 44±18       | 38.4±14.9   | 0.375   |
| Crea(mg/dl)     | 4.5±2.1     | 4.7±2.7     | 3.7±1.7     | 0.279   |
| GFR(ml/min)     | 18.7±9.3    | 21.9±13     | 26.3±13.8   | 0.034   |
| Dietary intake  |             |             |             |         |
| Vegetable(gr/day) | 71.7±13.5  | 66.3±13     | 67.2±17     | 0.312   |
| Dairy food(gr/day) | 43 ±9.7   | 37 ±11.4    | 39 ±12      | 0.065   |
| Meat(gr/day)    | 87±11.6     | 81.3±18     | 80±20       | 0.238   |
| Fruit(gr/day)   | 81.5±14     | 81 ±8.7     | 75.8±20     | 0.390   |
| Grains(gr/day)  | 38.7±8      | 36.7±8.5    | 38.3±7      | 0.706   |
| Others(gr/day)  | 510±330     | 476±258     | 405.6±128   | 0.508   |
| energy(kcal/day)| 1866±563.5  | 1838±233    | 2267±730    | 0.032   |
| Fat percent(%/E)| 36.7±9      | 38.7±9      | 37±9.5      | 0.465   |
| CHO.percent(%/E)| 50.3±10.3   | 51±6.6      | 57±16       | 0.171   |
| PRO.percent(%/E)| 10.7±2.3    | 11.2±2      | 12.2±2.6    | 0.069   |
Analysis was based on univariate general linear model (ANCOVA). First tertile was considered as reference tertile. M.D: Mean Difference, CI: Confidence Interval, Model I: adjusted for age and daily intake of energy, Model II: additional adjustment for gender, body mass index, diabetes and hypertension status.

According to the data of Table-5, in model I of adjustment, mean difference of creatinine in T₃ group compared to T₁ group of NEAP and NAE indexes was significant (P<0.01). That means by adjusting the confounders, the mean creatinine concentration in T₃ of NAE was significantly higher than the T₁. In the case of NAP, it was just the opposite; As the creatinine concentration decreased significantly across tertiles of NEAP (negative correlation). In crude model, mean difference of creatinine in T₃ group compared to T₁ group based on NEAP index was negatively significant (P<0.01). There was no significant difference in the model II adjustment of confounders.

Table 5: Mean Difference (M.D) of Creatinine across tertiles of PRAL, NEAP and NAE in CKD patients

| variable | tertile | Adjust model | M.D | P   | 95% CI | M.D | P   | 95% CI |
|----------|---------|--------------|------|-----|--------|------|-----|--------|
| GFR NAE  | T2      | crude        | 0.834 | 0.035 | 0.105 - 2.87 | -0.65 | 0.41 | -2.2 - 0.91 |
|          |         | Model I      | 2.25 | 0.014 | 0.47 - 4.02  | 10.6 | 0.004 | 3.5 - 17.7  |
|          |         | Model II     | 0.037 | 0.971 | -1.97 - 2.05 | 3.15 | 0.265 | -2.45 - 8.7  |
| PRAL     | T2      | crude        | 0.039 | 0.955 | -1.33 - 1.4  | 1.32 | 0.146 | -0.47 - 3.1  |
|          |         | Model I      | 7.44 | 0.009 | 1.97 - 12.9 | -1.7 | 0.229 | -4.5 - 1.1  |
|          |         | Model II     | 0.141 | 0.958 | -5.26 - 5.5 | -1.3 | 0.607 | -6.4 - 3.8  |
| NEAP     | T2      | crude        | -1.5 | 0.028 | -2.86 - 0.17 | -2.3 | 0.003 | -3.8 - 0.82 |
|          |         | Model I      | -2.46 | 0.097 | -5.4 - 0.46 | -5.44 | 0.007 | -9.35 - 1.52 |
|          |         | Model II     | 0.284 | 0.919 | -5.3 - 5.85 | -2.3 | 0.082 | -4.9 - 0.31 |

Analysis was based on univariate general linear model (ANCOVA). First tertile was considered as reference tertile. M.D: Mean Difference, CI: Confidence Interval, Model I: adjusted for age and daily intake of energy, Model II: additional adjustment for gender, body mass index, diabetes and hypertension status.

Discussion

The Iranian diet is thought to be relatively acidic due to its high consumption of refined grains such as white rice and non-alcoholic beverages (18). This type of conception was partially confirmed in our study because the values of the PRAL and NAE indices were inclined towards completely positive and acidic values. Of course, the average NAP values indicate the alkalinity of the diet of our patients. It should be noted that the PRAL index, despite its significant limitations, unlike the NEAP index, takes into account the amounts of potassium, magnesium and calcium in the diet. These ions play role in preventing acidification of the blood (24-26); Therefore, it seems that PRAL is more accurate. In Tehran Lipid and Glucose Study (16), mean of PRAL in
subgroup CKD patients was -19.46±1.5mEq/day; Indicates that the diet of Tehranian CKD patients was alkaline. In other study (27), mean value of NEAP of CKD patients was 50.1±13.1mEq/day. In Brazilian study (28), the median value of PRAL was 6.8 mEq/day and of NEAP was 53.1 mEq/day for CKD patients. In African American CKD patient (15), Median estimated NEAP was estimated 71 mEq/d. Another study (29) showed that median value of estimated DAL, is 47.24 mEq/d. The study among Venezuelan CKD children (30) indicated that mean of PRAL is 16 ± 10.7 mEq/day. In study of American CKD patients (31) mean NEAP, PRAL, and NAE were 58.2 ± 24.3, 9.7 ± 18.4, and 32.1 ± 19.8 mEq/day, respectively. Despite all studies were on CKD patients, differences in the results of these studies can be attributed to differences in population food patterns, age groups, designs and number of participants, measuring methods and food intake estimating method and variation in confounder variables.

Today's, due to the industrialization of the food production process and easier access to food and changing the people's tastes, consumption of refined carbohydrate-based foods, high sodium and protein foods, especially animal proteins has increased, while our ancestors ate plant-based foods and high fiber and potassium (32). This transition has changed very rapidly over the past decades. In patients with chronic renal failure due to the inability of the renal tubules to excrete toxic acidic metabolic products (15), the metabolic disorders resulting from this nutritional transition are exacerbated.

In our study, daily intake of energy and protein percent of energy in T₃ group of PRAL index were significant more than T₁; Also, the mean of daily energy intake increased significantly across the tertiles of NAE index. Other studies reported similar results (32). Eating protein-rich foods can increase the body's pool of amino acids and ultimately lead to an increase the free hydrogen ions in the body, which can lower the pH of the blood to an acidic state. It is also important to pay attention to the type of consumed protein. Protein from animal sources has high biological availability and is also high in phosphorus. The exception in animal sources is milk and dairy products, that the effect of high phosphorus, is neutralized by its high calcium content. In plant foods, phosphorus is mostly in the phytate form, which has low biological availability, so the acidifying effect of phosphorus in plant sources is greatly reduced (24-26). In addition, animal proteins are high in sulfur-containing amino acids, methionine and cysteine, which are converted to sulfuric acid in the body, so animal foods can increase the acidic load of body fluids through this physiological mechanism. Conversely, plant-based protein contains glutamine, Which is mostly known in the body as a consumer and recipient of hydrogen ions, so this is another mechanism for the positive effects of plant foods in improving acid–base balance in the body (24, 33). Vegetables are also high in potassium, which binds to organic anions and converts them to bicarbonate, thus slowing down the production of endogenous acid compared to animal foods (34). In the countries with nutritional transition, including Iran, is estimated that the share of animal protein is more than vegetable protein in diet and even this ratio is twice (35); From this point of view, a good outlook is not predictable.

In our study, mean of GFR increased significantly across the tertiles of NAE and NEAP indexes; This relationship seems irrational; But this significance was lost in general linear model test, and it is interesting that based on Crude General linear model, the higher tertiles of PRAL compared to first tertile had significant lower GFR. This difference was not significant in adjustment models; But, the third tertile of PRAL had less GFR than the first tertile. correlation of creatinine concentration with DAL was not seen after in crude model; After adjusting, the mean creatinine concentration in T₃ group of NAE was significantly higher than the T₁; In the
case of NAP, it was just the opposite; As the creatinine concentration decreased significantly across tertiles of NEAP (negative correlation). Other studies have shown that increasing the acid load of the diet is associated with low GFR and more reduction of it over the time and high Creatinine concentration (15, 27 and 36). A systematic review and meta-analysis of observational studies found that higher DAL could significantly increase the risk of CKD (37). Some cohort studies with follow-up periods of more than 10 years have shown the correlation of a high DAL with increased risk of progressing CKD (11, 20). Of course, some other cohort studies (38, 39) found no association between DAL and CKD disease. In one interventional study (40), effect of fruits and vegetables or bicarbonate in attenuation of kidney injury was concluded. The mechanisms of the effect of dietary acid load on the development of renal dysfunction can include: First, acidosis caused by high dietary acid load can increase amount of ammonium ions in kidney tissue without reducing bicarbonate levels, but can lead to toxic effects and damage to the tissue of the renal tubules, which in the long term reduces the function of the nephrons. This type of metabolic acidosis can also lead to increased production of endothelin, which reduces GFR and exacerbates renal tubular tissue damage. The second possible mechanism is increased production of oxygen free radicals and oxidative stress, which can lead to nephrotoxicity (41-44).

This is one of the limitations of studies such as our study that PRAL and NEAP estimating formulas is used to calculate the acidity, because these formulas do not account the biological availability of nutrients and sulfur content of different protein foods (46).

**Conclusion**

Finally, it can be said that indexes of dietary acid load include the PRAL, NEAP and NAE are related to renal function indices, but to understand the cause-and-effect relationships;

Because, while the validity of dietary acid load values calculated by PRAL formula versus it’s measured vale from urine has been confirmed (47, 48), due to the limited interpretation of the results obtained from the formulas for estimating the dietary acid load in different situations of urinary pH and blood bicarbonate concentration (28), long-term study with more and more population and taking into account blood bicarbonate concentration and urinary pH is proposed.

**Declarations**

**Authors’ contributions**

Research idea and study design: BE KOOR; data acquisition: MNJ, AM, YKH and PPA; data exploration: BE KOOR, MNJ, AM, YKH and PPA; statistical analysis and interpretation: Ali M and BE KOOR; Draft of manuscript: BE KOOR and Ali M. All authors read and approved the final manuscript.

**Ethics approval and consent to participate:**

The protocol of this study was reviewed and approved by the Ethics Committee of Yasuj University of Medical Sciences (Ethical code: IR.YUMS.REC.1396.29)). Written informed consent was obtained from participants. The study adhered to the tenets of the Declaration of Helsinki.

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Competing interests

There are no competing interests

Consent for publication

Not applicable

Availability of data and materials

The analysis dataset for the current study is available from the corresponding author on reasonable request.

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