# Estimation of Phenylalanine Hydroxylase Activity in Patients with Chronic Renal Failure

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**Abstract**

Renal failure is a serious disease that affects the kidney and leads to different chemical changes and most cases lead to death .The aim of the study is to measure the activity of phenylalanine hydroxyls (PAH), and biochemical variables (urea, creatinine, and albumin) In the blood and urine of healthy and chronic renal failure (CRF) individuals. The study included 80 patients aged between 78 and 20 years and 80 healthy subjects between the ages of 23-65 years. The results showed significant differences in the activity of PAH at $P \leq 0.0001$ in sera and urine chronic renal failure patients compared to healthy, as the of the activity significantly decreased in sera of patients with CRF and increased in urine. The mean activity of PAH was 54.67 ± 6.03 (IU / ml) in sera for CRF patients, whereas in healthy individuals it was 120.83 ± 23.21 (IU / ml), and the average activity of enzyme was 61.34 ± 7.12 in urine of patients with CRF compared with 33.87 ± 3.08 ( IU / ml) in healthy individuals. The activity of PAH enzyme was compared according to age groups, showing a decrease in the age group more than 45 years more than those in the age group less than 45 years with significant differences. In conclusion, the current study demonstrated that CRF is associated with loss of secretory function, and metabolism Endocrine actions in the kidneys, seems likely to lose renal activity phenylalanine hydroxylase.

***Keyword:*** *Chronic renal failure, phenylalanine, phenylalanine hydroxylase, renal function tests.*

## Introduction

It develops chronic kidney disease (CKD) is usually over many years, with a long latent period when the disease is clinically silent [1] , and is characterized by the gradual substitution of the structure of the natural kidney fibrous tissue. When these structural changes become apparent, it leads to a decline in the ability of the kidneys to process wastes in the blood and perform other functions. We knew the results of Kidney Disease Outcomes Quality Initiative (KDOQI) of the National Foundation for the kidneys (NKF) on chronic kidney disease (CKD) as damage to the kidney (structural or functional) or low filtration glomerular rate of less than 60 ml / min / 1.73 m2 for 3 months or more . KDOQI company also established a classification system of five stages for CRF, which primarily depends on the glomerular filtration rate [2]. During the early stages patients may show a normal or slight decrease in the rate of glomerular filtration (GFR) and albuminuria; later it progresses, leading to end-stage kidney disease (ESRD) or renal failure [3] . Diagnosis, evaluation and treatment mainly on the vital indicators that assess kidney function depends. Glomerular filtration rate (GFR) is still the ideal brand for kidney function [4]. Measuring GFR may take a long time, and therefore GFR is usually estimated from equations that take into account internal filtration markers such as serum creatinine and cystatin C. Other biomarkers such as albuminuria may precede decreased kidney function and have shown strong associations with disease progression. The results showed that new biomarkers appeared with a promise to detect kidney damage before the signs currently used [1].

An important enzymes that revealed modern clinical studies reported in patients with infections, inflammation and general immune activity is phenylalanine hydroxylase. The involvement of this data down regulating the activity of phenylalanine hydroxylase by oxidative stress in immune activation in vivo [5].

The activity of this enzyme was found in the liver, kidney[6,7]. and pancreas cells of mice, in a previous study, laboratory enzyme activity was measured in mouse tissues, was the majority for the effectiveness of phenylalanine hydroxylase in the liver. Both kidneys combined, 10% of total body enzyme activity, and pancreatic activity of phenylalanine hydroxylase formed a low fraction of total phenylalanine hydroxylase activity in the body [7].

Mammalian PheH is active as a homotetramer [8], each consists of three polypeptide monomer zones are the regulatory domain of the terminal N, and the domain's central catalyst, and the terminal tetramerization of the C domain containing the α-helix is used to tangle monomers [9]. The structures of the regulatory domains of PAH have an ACT domain [8], While the dimeric shape structure of the rat PAH contains both catalytic and regulatory domains but lacks the C-terminal remnants 24 needed to form a tetramer. Phenylalanine PAH activation involves a consensual change in which the remnants of the N-terminal move away from the active site [9] .

Phenylalanine hydroxylase (PAH) is a key enzyme in the catabolism and disposal of phenylalanine, therefore is responsible for serum Phe levels [10]. PAH stimulates the hydroxylation of L-phenylalanine (L-Phe) to L-tyrosine (L-Tyr), It is a mixed-function oxidase depends on the iron, used cofactor tetrahydrobiopterin (BH4) as a reducing agent to provide the two electrons needed for the reaction[11,12], by molecular division of O2, add one atom to the phenylalanine ring and convert the other into water [13]. This interaction in humans is vital to prevent the accumulation of neurotoxicity amounts of L-Phe [14,15].

## Materials and Method

**1- Subjects:** Between October 1st 2018 and April 31, 2019,160 subjects were recruited in this study, and 80 consecutive patients(37female & 43 male) diagnosed by expert physicians to be CRF from Murjan Medical City, plus 80 without kidney failure( 46 female & 34 male) as a control group.

**2- Sample collection:**

**Serum Preparation**

Five ml of blood was obtained from each subject by puncturing the vein in a sitting or lying position, then slowly pushed into disposable tubes containing a separating gel. Blood in the gel containing tubes was allowed to coagulate at room temperature and then centrifuge at 1000 x g for approximately 15 min and then the supernatant was obtained and stored at -20 ° C until analysis [8].

**Urine Preparation**

I attended a sample urine obtained in plastic packaging after being deposition of salts and impurities other by using the centrifuged at1000 cycles / minute (10 minutes) and directly measure to the activity of the enzyme.

**Methods Used**

The activity of PAH enzyme was measured based on Bublitze (1969) (154) method, which includes the addition of enzyme DMBH4 and the phenylalanine based on tyrosine in the presence of PAH enzyme. Orange Absorption measured at 450 nm wavelength. This absorption is directly proportional to the enzyme activity.

Solutions of the Materials Used: The phenylbenzene base substance (Phe) dissolved in the Tris HCl regulating solution to adjust pH = 7.2, Catalase enzyme solution (CAT), DL- Dithiotheritol Solution (DTT), DMPH4 solution (6,7-dimethyl-5,6,7,8-tetrahydropterine),Trich loroacetic acid (TCA), HNO3 20%, Reagent 1-Nitroso -2-naphtol (NNS) 0.1%, Standard tyrosine solution .

The concentration of urea was determined by the use of the diagnostic tool provided by CAM TECH MEDICAL and spectral method, Creatinine concentration is determined by Jaffe reaction by the use of Biolabo- Franc Diagnostic Kit and Serum albumin is administered by the use of a diagnostic kit equipped by the French company Biolabo.

The Lowry method [16] was used to measure protein concentration in urine and using Bovin serum albumin (BSA) as standard protein.

## Statistical Analysis

The data is calculated and analyzed using the Statistical Package for Social Sciences (SPSS) version

twenty of Windows. Data are expressed as (mean ± SD). Independent sample testing is used to compare means between two groups. Chi-square (X2) test is used to find the importance of separation variables. P values less than (0.05) are significant.

## Results

The study included (80) cases suffering from chronic renal failure by taking a blood and urine samples from patients, also included (80) samples of healthy persons as a comparison group. The overall mean age of patients with CRF and control were (60.16±13.20) and (57.12±12.71) years old, respectively. Table (1) shows the differences between patients with chronic renal failure and control by social and demographic characteristics.

**Table 1: Differences between patients and controls in residence, occupational status and educational status**

| Variable | Study Groups | | X2 | P-value | Odds ratio 95% C.I |
|---|---|---|---|---|---|
| | Patients% | Control% | | | |
| Gender<br>Female<br>Male | 34(42.5)<br>46(57.5) | 37(46.25)<br>43(53.75) | 0.227 | 0.633 | 0.633(0.623-2.173) |
| Residence<br>Urban area<br>Rural area | 55(68.75)<br>25(31.25) | 42(52.50)<br>38(47.50) | 4.424 | 0.035 | 0.036(1.044-3.794) |
| Occupational status<br>Employed<br>Non-Employed | 10(12.50)<br>70(87.50) | 20(25.00)<br>60(75.00) | 4.103 | 0.0428 | 0.046(1.014-5.371) |
| Educational status<br>Illiterate<br>Education | 35(43.75)<br>45(56.25) | 28(35.00)<br>52(65.00) | 1.283 | 0.257 | 0.258(0.366-1.309) |

P<0.05 is significant

Table (2) demonstrated the vital advantage in various chronic renal failure patients and control group, that there was a significant difference (P <0.001) in B. urea, creatinine S. albumin, as a significant difference (P <0.001) was recorded in the urine of patients and healthy people.

**Table (2): Biomarker level of (urea, creatinine, albumin)**

| Variables | | Normal Mean± SD | Patient Mean± SD | P- value |
|---|---|---|---|---|
| Urea (mg / dl) | Blood | 2.01± 23.16 | 67.42 ± 7.10 | < 0.001 |
| | Urine | 28.86 ± 1.05 | 13.10 ± 0.35 | < 0.001 |
| Creatinine (mg/dI) | Blood | 0.074±1.10 | 4.71 ± 0.31 | < 0.001 |
| | Urine | 3.21± 139.42 | 2.81 ± 0.08 | < 0.001 |
| Albumine (g/L) | Blood | 43.83 ± 12.02 | 2.73±25.05 | < 0.001 |
| | Urine | 0.179 ± 0.03 | 12.14 ± 1.28 | < 0.001 |

The activity of phenylalanine hydroxylase in the blood and urine of CRF patients and with healthy people was measured and compared using the Bublitze method (1969) [17] . When compared statistically, there were statistically significant differences between the activity of the enzyme in patients compared to the health level of probability $p \leq 0.001$ in the blood and urine, where the enzyme is less effective in patients with CRF in the blood while increasing the effectiveness of the enzyme in the urine as shown in Table (3) .

**Table (3): Activity PAH enzyme in blood and urine of CRF patients and healthy individuals**

| State | Activity PAH I.U / ml (mean ± SD) | P- value |
|---|---|---|
| Normal (Blood) | 120.83 ± 23.21 | < 0.001 |
| CRF (Blood) | 54.67 ± 6.03 | |
| Normal (Urine) | 33.87 ± 3.08 | < 0.001 |
| CRF (Urine) | 61.34 ± 7.12 | |

The activity of PAH enzyme in blood and urine in males and females according to different age groups has been studied. Tables (4) shows the mean and standard deviation of the effect of age and sex and their interaction on enzyme activity, the results showed that there were significant differences between patients and healthy individuals, as well as significant differences between the age groups of patients. When comparing healthy and chronic kidney failure patients by age group, the enzyme activity in the blood was decreased for both age groups (age <45 yr.) And (age> 45 yr.), and over 45 years of age were more than people in the age group less than 45 years, as Increased enzyme efficiency in diuresis has been shown for both groups, the rise of the enzyme PAH for those over 45 years older than the age group under 45 years.

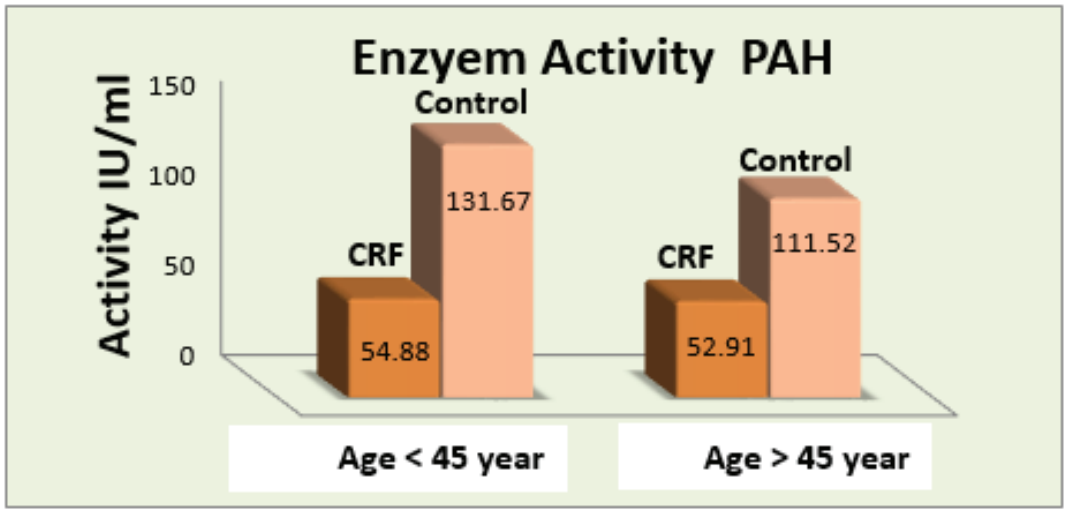


**Figure 1: Activity of PAH enzyme in blood for CRF patients and control by age group**

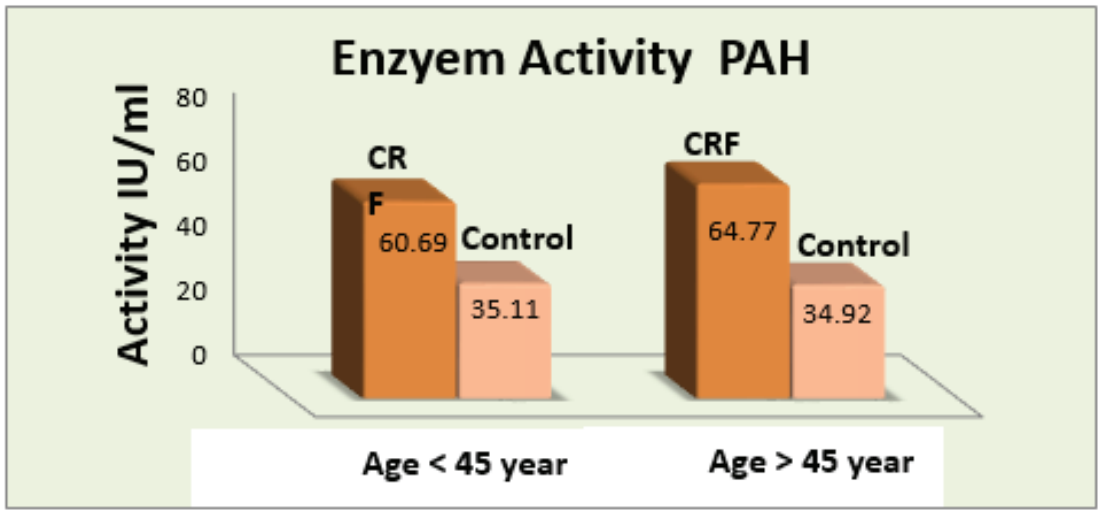


**Figure 2: Activity of PAH enzyme in urine for CRF patients and control by age group**

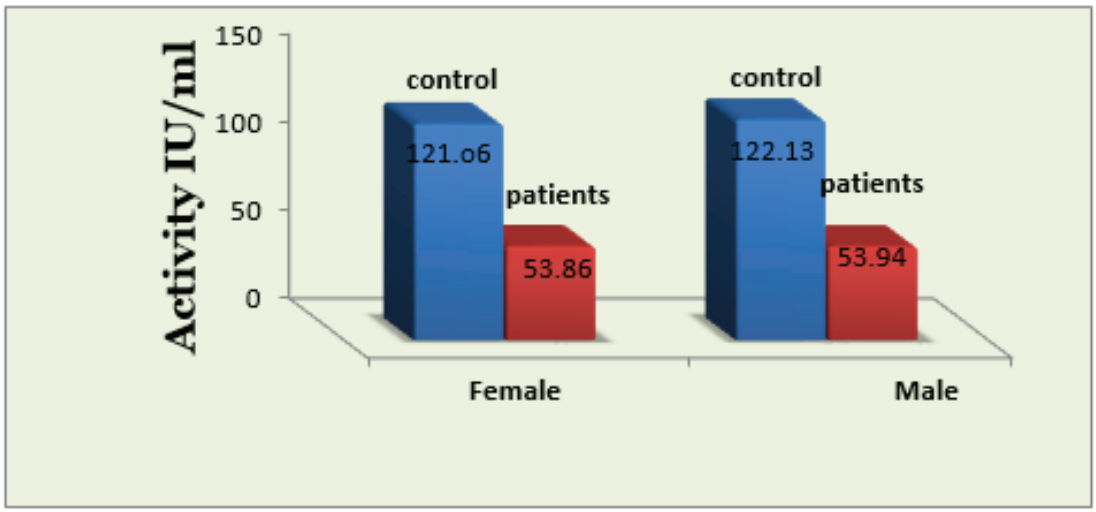


**Figure (3):Activity of PAH enzyme in the blood of patients with CRF compared to control group by sex**

## Discussion

The number of people with CRF in the city was higher in rural areas. This is due to several factors including environmental pollution[18,19] unhealthy eating habits, urban adoption of fast food [20,21], and canned foods [22]. In addition to psychological and economic factors, all these factors affect the occurrence of many diseases, including kidney failure[23,24] . This is consistent with the studies that have pointed to the high activity of the enzyme PAH in healthy blood and decline in urine and vice versa in patients with renal failure, as in the Diem K and his group (1970) [26] and Daphnis E and his group (2001) [27] in both males and females, as well as Pregnant women as in Lindheimer MD and his group (2001) [28]. The presence of significant differences between the age groups of patients corresponds to studies that showed that the most vulnerable to kidney failure are those aged between 50-67 years and men are more likely to develop kidney failure compared to women [29], unlike some studies that proved that women are more vulnerable The renal failure of men and the nature of the effectiveness of the kidney on glomerular filtration is less than men [30,31], Unlike some studies that have shown that women are more likely to have kidney failure in men and the nature of kidney effectiveness on glomerular filtration is lower than men [30]

## Conclusion

Our study provides data that patients with CRF impaired renal function may also alter PAH enzyme activity. However, results need to be confirmed by further investigation.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Al-Furat Al-Awast Technical University/ Babylon Technical Insituite, Iraq and all experiments were carried out in accordance with approved guidelines.

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