**IDENTIFICATION OF NADPH OXIDASE, COX-2, AND SOME BIOMARKERS IN RENAL FAULIR**

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**ABSTRACT**

Renal failure is increasingly being related to oxidative stress. For example, uremic patients have a lower antioxidant defense maximal range, which has been linked to oxidative stress (the generation of excessive reactive oxygen species (ROS)). The kidneys produce NADPH, a superoxide anion and a type of ROS. In renal failure patients, an increase in NADPH production adds to the development of renal damage. COX-2 is an enzyme that controls the flow of blood through the kidneys. When COX-2 inhibitors are used, kidney damage might occur, and renal blood flow can be negatively affected. This can then lead to the production of kidney failure. In this study, 60 samples of renal failure patients and 30 samples of healthy subjects of both genders, aged 15-25 years, were collected over four months. The assays were performed on NADPH Oxidase, COX-2, and other biomarkers (kidney functions, glucose, lipid profile, electrolyte (Na+1, K+1, Cl-1, Ca+2). As a result, we can confirm that the chemical evaluations revealed that the concentration of Serum COX-2 and NADPH can predict renal impairment. The results show rapid rise in serum levels of COX-2, NADPH in outpatients and inpatients with renal failure compared to control (p > 0.05). This investigation found that the increase in concentrations of both COX-2 and NADPH in the patient group was indicative of renal failure.

***Keywords: Renal failure, COX-2, NADPH OXIDASE, kidney functions, lipid profile, glucose, trace elements***

# **INTRODUCTION**

Renal failure happens when the kidney's capacity falls below 90% of what it was capable of. Renal failure's underlying cause is oxidative stress. Cellular processes such as tissue development, cell differentiation and proliferation, death, and modulation of blood vessel tone are regulated by reactive oxygen species. Renal injury is caused by an increase in ROS production. Patients with renal failure have been shown to have early oxidative stress. (Zhou et al. 2016) (Schröder 2019). Renal failure produces ROS as a result of proinflammatory and this increase in ROS results in oxidative stress. These factors then increase the probability of cardiovascular disease (CVD). A patient's pro-inflammatory condition in the presence of uremia may be exacerbated by ROS released by primed polymorphonuclear neutrophils (PMNs). Oxidative stress is the reason for the inflammatory milieu in renal failure (Betjes 2013). Vasoconstriction in the glomerular microcirculation is prevented by prostaglandins such as prostaglandin E2 (PGE2) and prostaglandin I2 (PGI2). Mesangial cell hypertrophy is promoted by Ang II through reactive oxygen species (ROS), which come from nicotinamide adenine dinucleotide phosphate and its reduced form (NADH/NADPH) oxidase, according to recent research. COX-2 expression and activity have been linked to renin-angiotensin system activation, according to previous studies in this area (Jaimes et al. 2005).

On the other hand, COX-2, which was previously assumed to be pathogenic, dramatically increases in expression when exposed to potentially damaging stimuli. As a result, homeostasis in the kidneys is maintained by the presence of this specific isoform. Understanding the essential biological role of COX isoforms will help researchers understand kidney physiology and disease and the consequences of pharmacologically suppressing an isoform's activity. (Goetz Moro et al. 2017)

Gene knockout experiments, on the other hand, found that COX-1 significant disruption had no influence on normal renal development. As with transgenic COX-2/ animals, a COX-2 selective inhibitor drastically slowed renal cortex development and decreased glomerular width during pregnancy, but a COX-1 selective inhibitor had no impact on renal development. There is a lot of evidence that NSAIDs and selective COX-2 inhibitors can affect the kidney in a variety of experimental and clinical situations, including how these drugs affect renal inflammation and water transport, as well as sodium and potassium balance, and how renal dysfunction or hypertension may result (Hörl 2010)

NADPH is used by all types of cellular life. The existence of mitochondria in eukaryotes is required for the production of NADPH in various other activities, which are all ROS producers in biological systems. Despite the fact that Nox2 is produced, Nox4 is the most prevalent in the kidney. Too far, studies have shown a connection between Nox4 overexpression and renal oxidative stress and pathological conditions such as diabetic nephropathy and chronic kidney disease (CKD) (Sedeek et al. 2013)

The mouse kidney expresses three different variants of NADPH oxidase's catalytic component (Nox1, Nox2, and Nox4). A streptozotocin-induced diabetic nephropathy model in ApoE2/2 mice reveals that the predominant source of renal ROS is Nox4. Reduction in albuminuria, structural integrity, and decreased glomerular damage were seen when Nox4 was eliminated, but not Nox1. An innovative small-molecule method for treating and/or preventing chronic kidney disease has been shown by these findings. Nox4 is a key generator of ROS required for renal damage in diabetes. The diabetic kidney is thought to have many ROS sources. (Jha et al. 2014)

Angiotensin II, mechanical strain, and inflammatory cytokines are all known to have a role in NADPH oxidase. Reactive oxygen species generation processes and the vascular consequences of oxidative stress are discussed in this review, which highlights the significance of oxidative damage in experimental and clinical hypertension (Briones and Touyz 2010) (Barton, Meyer, and Prossnitz 2019).

# **MATERIALS AND METHOD**

## **Materials**

The Nicotinamide adenine dinucleotide phosphate (NADPH RDEEH2401) and Cyclooxygenase-2 (COX-2 RDEEH1014) kits were purchased from the MyBioSource / USA, While Urea 2601, Creatinine 2639, Glucose 2586, Cholesterol 2580, Triglyceride 2577, High density lipoprotein HDL 2450, Electrolyte (Na, K, Cl) 0610761 and Calcium Ca +2 2541 were purchased from the Beckman Coulter / Ireland

## **Collection of blood samples**

The study includes 60 patients having Renal failure of both genders (female and male); their ages ranged (from 15-25) years. The samples were selected from patients who have been visiting the department of Dialysis at Al-Kindi teaching Hospital/ Baghdad-Iraq. The control group consisted of 30 volunteers of both genders where ages ranged (from 15-25) years. Blood specimens were freshly collected and allowed to be clotting at room temperature. Centrifugation was used to separate the serum within 15 minutes, and the resultant volume was divided into three equal pieces using a micropipette and stored at -20 °C before the biochemical test. Afterward, the 90 serum samples were evaluated against NADPH OXIDASE, COX-2 linked immune sorbent assay kits (Elisa), and other biomarkers including (urea, Creatinine Glucose Cholesterol Triglyceride High-density lipoprotein HDL, Electrolyte (Na, K, Cl Ca +2) using enzyme.

## **Biochemical Analysis**

### **Determination of Serum NADPH oxidase Activity**

Sniffer technology was employed to perform the experiment. Plates were coated before to use with the capturing antibody, whereas the biotin-conjugated antibody was employed for detection. As a further step, a buffer solution was used to wash out the wells and add the test samples, standards, and the biotin antibody from the conjugated detection system. Afterward, we added HRP-Streptavidin and a buffer was used to wash away the unbound conjugates. The TMB substrates have been used for visualizing HRP enzymatic reactions. HRP was catalyzed to TMB in order to produce a change in color from blue to yellow after the addition of an acidic solution. The intensity of the yellow color was proportional in amount to the target sample which was captured on the plate. The wavelength was measured with the O.D. absorbance at 450nm using the instrument ELISY Biotic / USA**.**

### **Determination of Serum COX-2 Activity**

Sandwich enzyme-linked immunosorbent assay technology was employed in the development of this kit. Plates with 96 wells the binding protein anti-COX-2 antibody has been utilized for detecting antibodies while the anti-COX-2 antibody was which was before. After that, the wells were rinsed with buffer solution, and the biotin antibody of the linked detection technique was added to all wells. Soluble conjugates were then swept away with wash buffer after HRP-Streptavidin was introduced. HRP enzyme processes can be observed using TMB substrate. The presence of an acidic solution changed the color of HRP from blue to yellow after it had been converted to TMB. The volume of COX-2 sample collected on the plate was directly related to the brightness of the yellow color. The wavelength was measured with the O.D. absorbance at 450nm using the instrument ELISY Biotic / USA.

### **Biochemistry full automated analyzer**

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| --- | --- | --- | --- |
| **Analytes** | **Principles** | **Wavelength** | **Equipment** |
| Glucose | UV enzymatic hexokinase method | 340 | Bechman coulter AU480 |
| Urea | Enzymatic (Urease, kinetic method) | 600 | Bechman coulter AU480 |
| Creatinine | Kinetic Jaffe method | 520 | Bechman coulter AU480 |
| Calcium | Calcium arsenazo method | 660 | Bechman coulter AU480 |
| Cholesterol | Enzymatic (cholesterol esterase) endpoint | 540 | Bechman coulter AU480 |
| Triglyceride | Enzymatic (Lipase) endpoint | 540 | Bechman coulter AU480 |
| HDL-Cholesterol | Enzymatic (cholesterol esterase, cholesterol oxidase, and a chromogen system) endpoint | 600 | Bechman coulter AU480 |
| Electrolytes  (Na, K, Cl) | Direct ion-selective electrodes | | Jocoh |

## **Statistical Analysis:**

Use of the computer software version of the statistical package for the social sciences was used to examine the data. The descriptive statistics of the SPSS program were used to analyze the data. A mean standard deviation was calculated using the mean value and the minimum and maximum values for each factor (SD). This study also used Pearson's correlation analysis in order to determine the relationships between all of the variables evaluated. It was determined that the statistical tests were fast at p0.05 with a 95% confidence range.

# **RESULTS AND DISCUSSION**

According to the result in Table‎ 1, the average age of the patient group (male) was (20.967±3.41), the female was (21.000±3.55) years old, and the control group was (19.607±3.9378) years old. According to the results, there was a statistically non-rapid rise in average patient age (P-value = 0.1101) when compared to the control group (P-value 0.05). as well as in BMI (Kg/m²) levels in Male (22.048±5.8) and Female (21.415±4.3) renal failure patients when compared with the control group (22.9±5.9).

**Table 1** Average Age and BMI of sample for patients with Renal Failure disease with control groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Anthropometric Measurements | Male with renal failure (30) | Female with renal failure (30) | (Control group). (30) | P value |
| Age (years) M ±SD | 20.967±3.41 | 21.000±3.55 | 19.607±3.9378 | 0.1101 |
| BMI(Kg/m²) M±SD | 22.048±5.8 | 21.415±4.3 | 22.9±5.9 | 0.104 |

The levels of serum COX-2 and NADPH activity in dialysis patients with renal failure and controls are shown in Table ‎2 as (ng/L) (mean ±SD). As a result, COX-2 and NADPH levels were considerably greater in renal failure (p 0.001), (P<0.001) were considerably higher than in the control group, were the Receiver Operative Characteristic curve (ROC curve) has been used to calculate the COX2 & NADPH levels ability have been showing a greater opportunity to predict renal impairment with significant sensitivity, and specificity.

**Table 2** The levels of serum COX-2 and NADPH activity and the precision and applicability of COX-2 and NADPH to renal failure with control groups.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Biomarkers** | **Male with renal failure (30) M ± SD** | **Female with renal failure (30) M ± SD** | **(Control group). (30)** | **ROC curve (AUC)** | **SE** | **Sensitivity** | **Specificity** | **P-value** |
| **COX-2**  **ng/L** | 0.860±0.7626 | 0.604±0.5127 | 0.517±0.4672 | 0.967 | 0.0758 | 93.2 | 50.0 | P=0.009 |
| **NADPH**  **ng/L** | 6.586±3.4175 | 10.685±5.3598 | 2.764±3.7881 | 0.967 | 0.0185 | 90.9 | 95.5 | P<0.001 |

**Table ‎3** shows the mean ± SD (mg/dL) and the precision and applicability of urea, creatinine, glucose, calcium, sodium, potassium, chloride, Cholesterol, Triglyceride, and HDL in serum of renal failure groups: patients and control.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biomarkers** | **Male with renal failure (30) M ± SD** | **Female with renal failure (30) M ± SD** | **(Control group). (30) M ± SD** | **SE** | **Sensitivity** | **Specificity** | **P-value** |
| **Urea** | 148.0± 29.95 | 134.92± 44.56 | 25.896±6.68 | 0.000 | 100.0 | 100.0 | P<0.001 |
| **Creatinine** | 8.95± 2.63 | 8.197± 2.00 | 0.671± 0.17 | 0.000 | 100.0 | 100.0 | P<0.001 |
| **Glucose** | 132.604±16.96 | 129.36± 12.01 | 95.114±9.2336 | 0.0683 | 40.4 | 91.7 | P=0.535 |
| **Ca II** | 7.83±1.31 | 8.24±1.21 | 8.99±1.47 | 0.0249 | 91.1 | 75.0 | P<0.001 |
| **Na+** | 146.13±22.67 | 150.05±14.27 | 143.27±12.14 | 0.0507 | 76.8 | 75.0 | P<0.001 |
| **K+** | 5.32±0.90 | 5.43±0.74 | 4.54±0.74 | 0.0437 | 80.4 | 92.9 | P<0.001 |
| **Cl-** | 98.41±8.86 | 95.84±18.24 | 100.79±14.11 | 0.0457 | 78.6 | 67.9 | P<0.001 |
| **Cholesterol** | 159.72±44.95 | 159.36±36.79 | 158.98±25.34 | 0.0609 | 71.7 | 23.3 | P=0.971 |
| **Triglyceride** | 91.970±28.45 | 122.251±35.11 | 87.258±25.3194 | 0.0621 | 76.7 | 33.3 | P=0.154 |
| **HDL** | 34.500±7.39 | 33.500±5.68 | 33.450±3.2 | 0.066 | 75 | 12.6 | P<0.001 |

This result congruent with (Goetz Moro et al. 2017) In patients with renal failure, COX-2 levels were found to be significantly higher than in the control group. Furthermore, Prostanoids with rapid biological relevance are exhibiting antiapoptotic capabilities, which play important roles in renal physiology. (Korbecki et al. 2014) PGI2 and PGE2 control blood circulation in the kidneys in reaction to variables that cause vasoconstriction, which affects the glomerular filtration rate in the case of blood volume depletion. (Meshram et al. 2021) according to these studies they believe that inflammation is the primary element that works in conjunction with a renal lesion to release a harmful process (Goetz Moro et al. 2017). Several studies revealed that research by (Kirkby et al. 2018) Microparticle depositing and pharmacologic COX-2 suppression were used to determine the role of COX-2 in localized blood supply in mice. COX-2 inhibition impacted all organs investigated, although renal blood supply was the most affected. spleen, adipose, and testes show only minor impacts. Local COX-2 expression was only seen in the kidney. COX-2 largely affects blood flow in this region. According to these results, renal blood flow is modulated by local COX-2. Only in the kidneys was it possible to identify local COX-2 expression. This is where COX-2 has the largest impact on blood flow. COX-2 inhibitors' kidney and cardiac side effects, as well as the potential for COX 2 as a targeted treatment in renal sickness, are directly related to these findings.

NADPH levels in the serum of renal failure patients were significantly elevated in this study. Serum NADPH levels rose rapidly in patients with renal failure as compared to those in a control group, according to studies by (Zhou et al. 2016) Renal failure patients have been shown to have an early oxidative stress. NOS inhibition causes kidney injury, proteinuria, and glomerulosclerosis in the long term. (Ito et al. 2013) In addition, hemodialysis patients had significantly higher levels of serum (NADPH) oxidase than those in the control group, according to research by (Yaribeygi et al. 2018). Nicotine adenine dinucleotide phosphate (NADPH) oxidation is the primary source of O2 in the artery wall and kidneys in hypertension animal models. Kidney function has been enhanced. ROS play a role in renal vascular constriction, renin release, and renal afferent stimulation neurons, heightened afferent arteriole contraction and myogenic responses, improved tubuloglomerular feedback, glomerular cell dysfunction, and proteinuria. (Rubattu et al. 2014).

(Uddin et al. 2021) The kidney is unable to correct for water-electrolyte, and acid-base imbalances, increasing OS. Finally, renal failure progresses, resulting in a range of problems. Because of this, a better way to treat renal insufficiency is needed. Numerous lines of evidence clearly suggest that oxidative stress (OS) plays a substantial role in the progression of renal failure, despite the fact that the exact mechanism is unclear. As a result, encouraging the body's antioxidant defense mechanism may become a key method for preventing OS-mediated cellular damage in renal failure. (Prieto-Bermejo and Hernández-Hernández 2017) A deeper knowledge of redox signaling during angiogenesis will enable the development of novel therapeutic techniques based on targeting the Nox family alone or in combination with other key components of this process.

The urea and creatinine markers showed a strong response to the renal failure disease, while Glucose results showed there was a non- rapid rise in serum level compared to control (p > 0.05). The studies congruent with a significantly increased in serum creatinine, and urea for dialysis patients with renal failure, which is the same as our study. (Batubo et al. 2020). The level of glucose shows an unexpected increase in serum of renal failure patients in this study. It is estimated that up to 40 percent of diabetic people have Diabetic Nephropathy, which is the major cause of renal failure illness. Diabetic nephropathy is caused by a variety of reasons (Hojs et al. 2020)

The calcium, Sodium and Potassium marker results showed a stronger response to renal failure disease, where is a rapid rise in serum levels of Sodium, as well as in Potassium serum levels for patients compared to healthy control. These results showed there was a rapid decrease in serum levels of Chloride and Calcium compared to control (p > 0.05). The remarkable data above is conducted to Congruent with Zhang et al. who reported a Rapid decrease in calcium serum in the renal failure patients compared to control. (Zhang et al. 2020). The results indicated that the levels of serum sodium were rapid rise in dialysis patients with renal failure. These results conducted with the result of (Canaud et al. 2019) as well as Serum potassium levels in the presents study were rapidly rise in dialysis patients compared to the control group (p < 0.05), these results Congruent with what (Canaud et al. 2019; Palmer and Clegg 2017) have discovered. Moreover, low levels of Cl have been seen rapidly in renal failure patients, which was in Congruent with the earliest study. (Mandai et al. 2017).

The cholesterol, triglyceride and HDL marker result has been showing a weak response to the renal failure disease. We found, the results for Cholesterol, triglycerides, and HDL-C levels showed a non- rapid rise between renal failure patients compared to the control group. Samouilidou et al. has been reported elevated levels of Cholesterol, high levels of triglycerides, and low serum levels of HDL-C. (Samouilidou et al. 2016) (p > 0.05).

This study also used Pearson's correlation analysis the result there was positive correlation between COX-2 with sodium and creatinine however there was no rapid correlation between COX-2 and other patients’ parameters as well as the positive correlation was between NADPH with patients’ serum BM1, sodium, potassium, urea, creatinine, glucose, and triglyceride however there was no rapid t correlation between NADPH and other patients’ parameters.

# **CONCLUSIONS**

Patients with renal failure who were compared to a control group had a significant increase in COX-2 activity levels, and this drop had a good AUC value. Serum levels of NADPH activity in patients with renal failure raised significantly control group with a high AUC value. In the diagnosis of renal failure, COX2 & NADPH could be used as useful sensitive markers.

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