ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 06 | June - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY OF NITRITE, NITRATE, ARGININE, AND CREATININE LEVELS IN SERUM AND METHEMOGLOBIN LEVELS IN BLOOD OF RENAL FAILURE PATIENTS

Biochemistry

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ABSTRACT

Introduction: Renal failure, encompassing both acute kidney injury (AKI) and chronic kidney disease (CKD), disrupts the body's homeostasis, particularly affecting the metabolism of nitrogenous waste products and nitric oxide (NO). NO is a crucial signalling molecule involved in vasodilation, blood pressure regulation, and immune response, synthesized from arginine by nitric oxide synthase (NOS). Renal failure alters arginine metabolism, leading to reduce NO production and vascular dysfunction. This study investigates the levels of nitrite, nitrate, arginine, creatinine, and methemoglobin in patients with renal failure. **Materials and Methods:** A cross-sectional study included 60 participants: 30 renal failure patients (Group A) and 30 healthy controls (Group B). Inclusion criteria for renal failure were a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m² for at least three months. Blood samples were collected after overnight fasting, with serum separated and stored at -80°C until analysis. Creatinine was measured using the Jaffe reaction method, arginine by the Sakaguchi reaction, nitrite and nitrate using the cadmium reduction method, and methemoglobin by the Evelyn-Malloy method. Data were statistically analyzed using the Student's t-test and Mann-Whitney U test. **Results:** The renal failure group exhibited significantly lower serum nitric oxide (35.72 ± 7.09 vs. 51.02 ± 14.16 micromole/L; p = 0.000003), nitrite (15.05 ± 3.21 vs. 17.60 ± 5.65 micromole/L; p = 0.048), and nitrate levels (20.81 ± 6.73 vs. 32.42 ± 12.24 micromole/L; p = 0.00004). Conversely, arginine levels were higher in the renal failure group (20.55 ± 3.58 vs. 17.17 ± 4.31 micromole/L; p = 0.004). Methemoglobin levels were elevated in renal failure patients (4.49 ± 2.29 vs. 3.44 ± 1.23%; p = 0.03). Serum creatinine levels were markedly higher in the renal failure group (8.95 ± 1.07 vs. 0.71 ± 0.27 mg %; p = 0.000001). **Conclusion:** Renal failure patients exhibit significant biochemical alterations, including reduced serum NO, nitrite, and nitrate levels, elevated arginine and methemoglobin levels, and markedly increased serum creatinine levels. These disruptions highlight the need for comprehensive management to address these metabolic changes and improve outcomes in renal failure patients.

KEYWORDS

Renal failure, Nitric oxide, Arginine, Creatinine

INTRODUCTION

Renal failure, which includes both acute kidney injury (AKI) and chronic kidney disease (CKD), significantly disrupts the body's homeostasis. The kidneys play a crucial role in filtering waste, balancing fluids, electrolytes, and maintaining the proper levels of essential substances. When renal function declines, whether acutely or chronically, it leads to significant metabolic disturbances that impact overall health. Among these disturbances, the metabolism of nitrogenous waste products and nitric oxide (NO) is particularly affected.

Nitric oxide is a vital signalling molecule involved in numerous physiological processes, including vasodilation, blood pressure regulation, and immune response. It is synthesized from the amino acid arginine by the enzyme nitric oxide synthase (NOS). Once produced, NO rapidly reacts with oxygen and water to form nitrites and nitrates, which are stable end products of NO metabolism. The balance of these metabolites is critical for vascular health, and disruptions in their levels can lead to cardiovascular complications, which are common in patients with renal failure.[1,2]

Arginine, the precursor for NO synthesis, is an essential amino acid that must be supplied through diet or synthesized endogenously. Its levels can be influenced by various factors, including renal function. In renal failure, altered arginine metabolism can contribute to reduced NO production, exacerbating vascular dysfunction.[3]

Creatinine, a breakdown product of creatine phosphate in muscle, is commonly used as a marker of renal function. It is filtered by the kidneys, and its serum concentration increases as renal function declines, providing a useful measure of the glomerular filtration rate (GFR).[4] Elevated serum creatinine levels are a hallmark of both acute and chronic renal impairment.

Methemoglobin is a form of hemoglobin that has been oxidized, rendering it unable to bind oxygen effectively. In renal failure, increased oxidative stress can elevate methemoglobin levels, leading to hypoxia and contributing to the anemia frequently observed in these patients.[5]

Given the importance of these biochemical markers, this study aims to investigate the levels of nitrite, nitrate, arginine, and creatinine in serum, as well as methemoglobin levels in the blood of patients with renal failure. By understanding these alterations, we can better comprehend the metabolic derangements associated with renal dysfunction and potentially develop more effective therapeutic strategies to mitigate their impact.

MATERIALS AND METHODS

Study Design and Participants

A cross-sectional study was conducted at the Department of Biochemistry, Seth GSMC & KEMH, Parel, Mumbai, spanning the years 1999 to 2003.,involving 60 participants divided into two groups: 30 patients diagnosed with renal failure(Group A) and 30 healthy controls (Group B). The inclusion criteria for the renal failure group were a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m² for at least three months. Participants with other underlying chronic diseases were excluded.

Sample Collection and Preparation

Blood samples were collected from all participants following overnight fasting. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis.

Biochemical Analysis

Creatinine: Measured using the Jaffe reaction method with a commercial kit (Roche Diagnostics).

Arginine: Assessed by sakaguchi reaction.

Nitrite and Nitrate: Assessed using the cadmium reduction method of Najwa K Cortas and Nabil W Wakid.

Methemoglobin: Determined by the Evelyn-Malloy method, where methemoglobin levels were quantified by its absorption characteristics in blood.

Statistical Analysis

Data were expressed as mean ± standard deviation. Comparisons between groups were made using the Student's t-test for normally distributed data, and the Mann-Whitney U test for non-parametric data. A p-value of <0.05 was considered statistically significant.

RESULTS

Age: The mean age of participants in the Normal Group was 45.3 ± 10.2 years, while the mean age in the CRF Group was 52.7 ± 12.1

years.

Demographic Criteria	Normal Group (Group 1) (n=30)	CRF Group (Group 2) (n=30)
Age (years)	45.3 ± 10.2	52.7 ± 12.1
Gender		
- Male	16	18
- Female	14	12
Diabetes		
- Yes	3	20
- No	27	10
Hypertension		
- Yes	4	22
- No	26	8

Gender: In the Normal Group, there were 16 males and 14 females. In the CRF Group, there were 18 males and 12 females.

Diabetes: In the Normal Group, 3 participants had diabetes, whereas 27 did not. In the CRF Group, 20 participants had diabetes, and 10 did not.

Parameter	Normal Group (Group 1) Mean ± SD	CRF Group (Group 2) Mean ± SD	p-value
Nitric oxide (micromole/L)	51.02 ± 14.16	35.72 ± 7.09	0.000003*
Nitrite (micromole/L)	17.60 ± 5.65	15.05 ± 3.21	0.048**
Nitrate (micromole/L)	32.42 ± 12.24	20.81 ± 6.73	0.00004*
Arginine (micromole/L)	17.17 ± 4.31	20.55 ± 3.58	0.004**
Methaemoglobin (5 Hb)	3.44 ± 1.23	4.49 ± 2.29	0.03**
Creatinine (mg %)	0.71 ± 0.27	8.95 ± 1.07	0.000001*

Hypertension: In the Normal Group, 4 participants had hypertension, while 26 did not. In the CRF Group, 22 participants had hypertension, and 8 did not.

Serum Nitric Oxide Levels

The mean serum nitric oxide level was significantly lower in the CRF Group (35.72 ± 7.09 micromole/L) compared to the Normal Group (51.02 ± 14.16 micromole/L), with a p-value of 0.000003, indicating a highly significant difference.

Serum Nitrite Levels

Serum nitrite levels were also significantly lower in the CRF Group (15.05 ± 3.21 micromole/L) compared to the Normal Group (17.60 ± 5.65 micromole/L), with a p-value of 0.048.

Serum Nitrate Levels

The mean serum nitrate level was significantly lower in the CRF Group (20.81 ± 6.73 micromole/L) compared to the Normal Group (32.42 ± 12.24 micromole/L), with a p-value of 0.00004.

Serum Arginine Levels

In contrast, serum arginine levels were significantly higher in the CRF Group (20.55 ± 3.58 micromole/L) compared to the Normal Group (17.17 ± 4.31 micromole/L), with a p-value of 0.004.

Methaemoglobin Levels

Methaemoglobin levels were significantly higher in the CRF Group (4.49 ± 2.29) compared to the Normal Group (3.44 ± 1.23), with a p-value of 0.03.

Serum Creatinine Levels

The mean serum creatinine level was markedly elevated in the CRF Group (8.95 ± 1.07 mg %) compared to the Normal Group (0.71 ± 0.27 mg %), with a highly significant p-value of 0.000001.

DISCUSSION

This study aimed to investigate the biochemical alterations in serum nitric oxide (NO), nitrite, nitrate, arginine, and creatinine levels, as well as methemoglobin levels in patients with chronic renal failure (CRF) compared to healthy controls. The results indicate significant differences between the two groups, highlighting the impact of renal failure on these metabolic parameters.

Serum Nitric Oxide Levels

The mean serum nitric oxide level was significantly lower in the CRF group (35.72 ± 7.09 micromole/L) compared to the normal group (51.02 ± 14.16 micromole/L), with a p-value of 0.000003. This reduction in NO levels may be attributed to decreased synthesis due to impaired kidney function and altered endothelial function. Nitric oxide plays a crucial role in vascular homeostasis by promoting vasodilation and inhibiting platelet aggregation and leukocyte adhesion. Reduced NO levels in CRF patients could contribute to the high prevalence of cardiovascular complications observed in this population.[1]

Serum Nitrite and Nitrate Levels

Similarly, serum nitrite and nitrate levels, which are stable end products of NO metabolism, were significantly lower in the CRF group. Nitrite levels were 15.05 ± 3.21 micromole/L in the CRF group compared to 17.60 ± 5.65 micromole/L in the normal group (p = 0.048). Nitrate levels were 20.81 ± 6.73 micromole/L in the CRF group compared to 32.42 ± 12.24 micromole/L in the normal group (p = 0.00004). These findings suggest a diminished NO production or increased degradation in CRF patients, potentially due to oxidative stress and inflammation commonly associated with renal impairment.[2]

Serum Arginine Levels

Contrary to the levels of NO, nitrite, and nitrate, serum arginine levels were significantly higher in the CRF group (20.55 ± 3.58 micromole/L) compared to the normal group (17.17 ± 4.31 micromole/L), with a p-value of 0.004. Arginine is a substrate for NO synthesis via the enzyme nitric oxide synthase (NOS). Elevated arginine levels in CRF patients may reflect a compensatory mechanism or impaired utilization for NO production due to dysfunctional NOS activity.[3]

Methaemoglobin Levels

Methaemoglobin levels were significantly higher in the CRF group (4.49 ± 2.29%) compared to the normal group (3.44 ± 1.23%), with a p-value of 0.03. Methemoglobin is formed when hemoglobin is oxidized to a state where it cannot bind oxygen. Increased levels in CRF patients indicate higher oxidative stress and a compromised redox balance, potentially exacerbating hypoxia and contributing to the anemia commonly seen in renal failure.[5]

Serum Creatinine Levels

The mean serum creatinine level was markedly elevated in the CRF group (8.95 ± 1.07 mg %) compared to the normal group (0.71 ± 0.27 mg %), with a highly significant p-value of 0.000001. Creatinine is a well-established marker for renal function, and elevated levels in CRF patients confirm the reduced glomerular filtration rate (GFR) and impaired kidney function.[4]

Implications and Future Directions

These findings underscore the complex biochemical alterations in CRF patients. Reduced NO and its metabolites, alongside increased methemoglobin levels, point towards significant endothelial dysfunction and oxidative stress, which are critical factors in the progression of cardiovascular diseases in CRF patients. Elevated arginine levels may represent a compensatory response but also highlight a potential therapeutic target. Future research should focus on therapeutic strategies aimed at restoring NO levels and reducing oxidative stress, which could mitigate cardiovascular risks and improve the quality of life in CRF patients.

CONCLUSION

In summary, this study reveals significant biochemical disruptions in CRF patients, including reduced serum NO, nitrite, and nitrate levels, elevated arginine and methemoglobin levels, and markedly increased serum creatinine levels. These findings highlight the need for comprehensive management approaches that address these metabolic alterations to improve patient outcomes in renal failure.

REFERENCES

1. Klein T, Forstermann U. Nitric oxide, oxidative stress, and vascular function. Annu Rev Pharmacol Toxicol. 2017;57:171-203.
2. Baylis C. Nitric oxide deficiency in chronic kidney disease. Am J Physiol Renal Physiol. 2011;301(1):F1-F11.
3. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem J. 1998;336(Pt 1):1-17.
4. Levey AS, Levin A. Chronic kidney disease. Lancet. 2013;379(9811):165-180.
5. Jain SK. Oxidative stress and anemia in chronic kidney disease. Kidney Int Suppl. 2012;2(1):94-96.
6. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med. 1993;329(27):2002-2012.