زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

# Biochemical and Hematological Parameter Evaluation in a Patients with Renal Failure undergoing Dialysis

Research project

Submitted to the department of (Biology) in partial fulfillment of the requirements for the degree of BSc. In (Biology)

By

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Supervised by

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# SUPERVISOR CERTIFICATE

This project research has been written under our supervision and has been submitted for the award of the degree of Bachelor of Science in **Biology/Blood Physiology** with our approval as supervisor.

Signature Name: **Asst. Lecturer Darya Mohammed Aziz**

Date

**I confirm that all requirements have been fulfilled.**

Signature:

Name: **Mr. Mohammed Ali Saleem**

Head of the Department of Biology

Date:

# DECLARATION

I declare that the Bachelor project research entitled:

**Evaluation in a Patients with Renal Failure undergoing Dialysis** is our own original work, and hereby certify that unless stated, all work contained within this project research is our own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Signature:

Student: Shadi Osman & Lima Hakim

Date: / /2024

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# Dedication:

This research paper is dedicated to my parents who gives their out most support, and never-ending inspiration throughout the study. It also dedicated the teachers who supported us to reach to this level of education and are behind of this success. We look up and dedicated this whole study to our Almighty God who gave the strength, knowledge, wisdom,protection, and will to continue and keep positive to finish this research.

# Content:

# **Introduction**:

The condition when the kidneys lose their normal functionality called renal failure . It occurs when the kidneys cannot properly remove wastes that cause a buildup of waste and fluid in the body

In the other way, renal failure is characterized by a wide change of biochemical instabilities and many clinical symptoms and signs.

### 1.1-Dialysis therapy for chronic kidney disease

Dialysis is the preferred way to treat end-stage renal disease (ESRD )and remove accumulated toxins from the body.Dialysis is the process of removal of waste and extra water from blood.

Dialysis it is an artificial replacement of kidney functioning, especially in renal failure cases. Dialysis cannot completely perform lost kidney function, but, to some extent, manages its activities by means of diffusion and ultrafiltration.

### 1.2-Types of dialysis

There are two ways to get dialysis: Peritoneal dialysis and Hemodialysis. peritoneal dialysis (using a peritoneal membrane as a filter). Peritoneal dialysis is recommended for younger patients because of its flexibility and can be performed at home. In hemodialysis, blood is pumped out of your body to an artificial kidney machine, and returned to your body by tubes that connect you to the machine.

### 1.3-The mechanism of hemodialysis

The mechanism of hemodialysis is the wastes and excess water are removed by using an external filter called a dialyzer, which contains a semipermeable membrane. The separation of wastes is done by creating a counter-current flow gradient, where blood flow is in one direction and the fluid of the dialyzer is in the opposite direction.

### 1.4-Principle of Hemodialysis

The basic principle involved in dialysis is the movement or diffusion of solute particles across a semipermeable membrane (diffusion). Metabolic waste products, such as urea and creatinine, diffuse down the concentration gradient from the circulation into the dialysate.

### 1.5-Factor that affects of dialysis efficiency

The primary goal of hemodialysis is to restore the intracellular and extracellular fluid environment that is characteristic of normal kidney function . The difference in the time of dialysis depends on various factors, including kidney function, amount of waste in body, level of salts and body weight. Hemodialysis can be done at a dialysis center or at home. Treatments usually last about four hours and are done three times per week. Some people may need more time for treatments based on their specific needs.

**1.6-Impact of regular routine**

Most people on dialysis are able to keep a regular routine except for the time needed for treatments. Dialysis often makes people feel better because it helps clear the waste products that have built up in the blood between treatments. However, some people report feeling tired after dialysis, especially if they have been getting dialysis treatments for a long time

## material and methods

The research was carried out in kidney dialysis center and laboratory to analyze various changes in biochemical and haematological tests with chronic kidney failure patients. Here below a detail of area of study of time, sample collection and data analysis all discussed in materials and methods with proper methods.

**2.1-Area and time of study:**

A descriptive study was conducted at Kidney dialysis center/Erbil training Hospital ,from November 2023 to December 2023.This hospital is main dialysis center in Erbil, it has 350 patients approximately.

information to all patients such as chronic kidney failure. This hospital receives a lot of samples from all diseases associates with kidney and its treatment , But because laboratory facilities wasn't available, we had to take samples to a laboratory out of the hospital , in order to perform laboratory tests specialized to Kidney dialysis.

**2.2-Collection of samples:**

Samples from 42 patients of Chronic Renal Failure were collected. 2-3 ml of peripheral venous blood was drawn using standard procedure.1.0 ml of blood was transferred into a test tube containing dried EDTA for complete blood count and using Hematology Cell Counter (Coulter ). Other 2.0 ml blood transferred into a test tube with no anticoagulant for biochemical tests using (cobass )

Sample collection is done by trained nurses working in hospital and lead to proper testing for further analysis.

**2.3-Complete Blood Measurements:**

Complete blood counts (CBC) of all blood samples were carried out at Nanakaly hospital Laboratory. The blood parameters included total white blood cells count (WBCs), platelet count (PLTs), hemoglobin (Hbs), packed cell volume (PCV), were measured by coulter counter (Sysmex K-1000), TOA medical electronics CO., LTD. KOBE. JAPAN (Haen, 1995 and Rodak, 1995).

**2.4-Differential Leukocyte Percentage and Count (DLC):**

The leukocyte types were determined by preparation of blood films. A drop of (EDTA blood) was placed about 2 to 3 mm at one end of a clean slide. Place the pusher slide at 30$^{o}$ to 45$^{o}$ angle to the slide, and then move it back to make contact with the drop. The drop should spread along the edge of the pusher slide, and a film is made by a forward movement of the pusher slide. Blood films should be air – dried as soon as they were made. The blood films were fixed with undiluted stock Lei-shaman stain for 2 to 3 minutes, then the stain on film was diluted with distilled water, and left for 5-7 minutes, and then washed by a stream of tap water, the stained film was dried and examined under light microscope (Oil - immersion objective) employing a battlement counting technique, and at least (200) consecutive leukocytes should be identified (Evatt *et al*., 1992).

**2.5-Biochemical analysis:**

Serum biochemical analyses were determined using (Cobas C 311) full automatic.
These tests included total protein (TP: mg/dl), glucose (GLO: mg/dl), Lipid profiles (Cholesterol (CHO: mg/dl), Triglycerides, HDL, LDL, GPT, GOT, Alkaline phosphatase, total serum bilirubin and direct bilirubin.

One ml of the serum was added to flexor tube and serum glucose, total cholesterol, triglyceride (TG) high density lipoproteins (HDL) concentration, total protein, AST, ALT, Alkaline phosphatase cratnin, uric acid , urea, total serum bilirubin and direct bilirubin concentration is analyzed by using automatic chemical analyzer (Cobas C 311 biochemical analyzer) use only 20 µL of serum for each test.

**2.6-Determination of Low Density Lipoproteins (LDL)**

The lipid profile does not measure LDL level directly but instead estimates it via the Friedewald equation: LDL Cholesterol (mg/dl) = (Total Cholesterol - HDL Cholesterol – VLDL)
When VLDL = triglycerides (mg/dl)/ 5.

**2.7-Statistical Analysis:**

All data are expressed as means ± standard error (M ± SEM); statistical analysis of the obtained data was done according to independent simple's t-test and correlation was used to compare the means. The statistical analysis was carried out using statistically available software (Graphpad version 8).

## Results & Discussion

The haematological and biochemical results obtained from this study are summarized in Tables (1,2&3).

### 3.1- Impact of dialysis on RBC profile:

Hematological parameters including Hb, RBCs,Hct, RDW and Platelets were measured selected male and female of dialyzed patients and compared with healthy population.
In this study the total RBC count showed a significant increase after dialysis in all patients
In our study, Hb level was found to be increased after hemodialysis as compared to pre-dialysis. Mean Hb value in pre- dialysis patients was 9.7(8.9-11) whereas in post dialysis patients, it was 11.2(9.55-12.6)
Platelets were found to be decreased after dialysis as compared to pre- dialysis. HCT level after dialysis was increased when compared to predialysis cases. Mean value of HCT before dialysis was 31.2(27.75-34.8) whereas after dialysis it was 35.2 (31.25-40.7). Our study shows that most of the hematological parameters increased after a dialysis session. Therefore, it is recommended that all patients be screened appropriately before and after dialysis to avoid complications.and this is in agreement with findings of (Ravi Gautam and Prakash M. Patil 2018).

Table (1) Show RBC profile complications in chronic kidney frailer patients.

| Parameters | Patient | | P-Value |
|---|---|---|---|
| | Before | After | |
| RBC (10*12/l) | 3.28(2.93-3.81) | 3.77(3.29-4.45) | ⬆ 0.001 |
| HB (g/dl) | 9.7(8.9-11) | 11.2(9.55-12.6) | ⬆ 0.001 |
| HCT (%) | 31.2(27.75-34.8) | 35.2(31.25-40.7) | ⬆ 0.001 |
| RDW (%) | 12(10.8-12.75) | 12.2(11-12.7) | NS |
| PLt | 159(122-217) | 157(118-212) | NS |

**3.2- Impact of dialysis on RBC profile:**

Table 2 shows the effect of dialysis on different Leukocyte counts, our result showed that each of absolute Lymphocytes and Mid showed a significant increase after dialysis process in chronic kidney patients when compared with before getting treatment. In our study WBC level was found to be increased after dialysis when compared to pre- dialysis patients. Mean WBC value in pre- dialysis patients was 5.5(4.7-6.85) whereas in post dialysis patients, it was 5.8(5-7.7) with a significant p value of 0.01.An increase in white blood cells (leukocytosis) after dialysis in individuals with kidney failure can occur due to several reasons:

Inflammatory Response: Kidney failure is often associated with chronic inflammation. During dialysis, the body may respond to the stress of the procedure by releasing inflammatory cytokines, which can stimulate the production and release of white blood cells from the bone marrow into the bloodstream.

Dialysis-Related Factors: Dialysis itself can trigger an inflammatory response. The contact of blood with the dialysis membrane, exposure to dialysis solutions, and activation of the complement system can all contribute to inflammation and the subsequent mobilization of white blood cells.

Infection: Infections are relatively common in individuals with kidney failure, especially those undergoing dialysis. The presence of an infection can lead to an increase in white blood cells as the body responds to the invading pathogens. Infections can occur in various sites, including the access site for dialysis (e.g., catheter site infection), urinary tract, or respiratory tract.

Tissue Injury: Dialysis can sometimes cause tissue injury, particularly if there are issues with vascular access or if the procedure is not well-tolerated by the patient. Tissue injury can trigger an inflammatory response and lead to an increase in white blood cells.

Underlying Conditions: Individuals with kidney failure may have other medical conditions or comorbidities that can contribute to leukocytosis, such as autoimmune diseases, malignancies, or other inflammatory conditions.

Medications: Some medications used in the management of kidney failure or its complications can affect white blood cell counts. For example, certain immunosuppressive drugs or growth factors may influence the production or function of white blood cells.

In a study of Pandian J et al, 13 WBC count was decreased after dialysis when compared to pre- dialysis cases. In a study of Mohd Ali MS et al, 10 WBC count was found to be increased in post dialysis patients when compared to pre- dialysis patients.(Pandian J, Amit Kumar K, Swaminathan A. 2017;26:213-218)

In a study of Hakim Y AH et al, 12 WBC count was increased in post dialysis cases when compared to predialysis patients. (Mohamed Ali MS, Babiker MA, Merghani LB, et al.2008;19(2):274-279)

Table (2) Show Leukocytes profile complications in chronic kidney frailer patients.

| Parameters | Patient | | Patient |
|---|---|---|---|
| | Before | After | |
| WBC | 5.5(4.7-6.85) | 5.8(5-7.7) | ↑ 0.01 |
| LYM# | 2.868±0.229 | 3.427±0.264 | ↑ 0.01 |
| LYM% | 51.3(31.4-79.4) | 56.9(31.55-77.45) | NS |
| MID# | 0.6(0.45-0.8) | 0.7(0.5-0.95) | ↑ 0.05 |
| MID% | 10.4(7.9-13.3) | 10.8(7.85-13.2) | NS |
| GRA# | 2.05(0.425-3.6) | 1.65(0.5-3.7) | NS |
| GRA% | 2.05(0.425-3.6) | 1.65(0.5-3.7) | NS |

**3.3-Some biochemecial changes in pre and post dialysis:**

In chronic renal failure there is a steady and continued decrease in renal clearance or glomerular filtration rate (GFR), which leads to the gathering of urea, creatinine and other waste metabolites in the blood. Haemodialysis is considered as a good therapeutic option in the context of the renal replacement therapies in which different body waste products including urea, creatinine and free water are removed from the blood.Table 3 illustrate to some biochemical changes in response to dialysis when compared with pre ones

**3.4-Impact of dialysis on lipid profile:**

According to our result the lipid profile showed a slight increase in post dialysis when compared with pre dialysis the cholesterol level increased form (173.7±22.5) to (215.8±21.84) and LDL showed slight elevation with p value (0.001) in all patients after treatment, while albumin level showed no significant difference in both groups these results were accordance to that found by (Khaled MD, Faiz A, Abdelgader AT, et al.(2015);4:45-54-Sathiyanarayanan, S., Shankar M.P. and Prabhakar, E.R. (2013)64(87): 92–98.). There are a few reasons why lipid profiles may increase after dialysis:

Dialysis Clearance: Dialysis removes waste products and excess fluids from the blood, but it may not be as effective in removing lipids, particularly certain types of lipids like cholesterol. Therefore, while dialysis helps in managing kidney failure, it might not address lipid levels as effectively, leading to their increase post-dialysis.

Disrupted Lipid Metabolism: Kidney failure can disrupt lipid metabolism in various ways. For example, impaired kidney function can lead to changes in hormone levels (such as insulin and thyroid hormones) and enzyme activity, which in turn can affect lipid metabolism. These disruptions may persist even after dialysis, contributing to increased lipid levels.

Inflammation and Oxidative Stress: Kidney failure is often associated with chronic inflammation and oxidative stress, both of which can influence lipid metabolism and contribute to dyslipidemia (abnormal lipid levels). Dialysis may alleviate some of these issues but may not completely reverse the underlying inflammatory and oxidative processes.

Changes in Lipid Transport Proteins: Kidney failure can affect the levels and function of proteins involved in lipid transport, such as lipoproteins. These changes can persist despite dialysis and contribute to alterations in lipid profile.

Dietary Factors: Dialysis patients often have dietary restrictions, including limitations on potassium, phosphorus, and sodium intake. These dietary changes may affect lipid metabolism and contribute to alterations in lipid levels post-dialysis.

Medications: Patients undergoing dialysis often take medications to manage various aspects of their condition, including lipid-lowering drugs such as statins. However, the effectiveness of these medications may vary, and lipid levels may still fluctuate despite treatment.

### 3.5-Impact of dialysis on albumin:

The results of normal total protein concentration were not inconsistency to those results obtained by other studies  Etiology of the Protein-Energy Wasting Syndrome in Chronic Kidney Disease : A Consensus Statement from the International Society of Renal Nutrition and Metabolism (ISRNM). J. Ren. Nutr., 23(2): 77– 90.], they found that there was a non-significant difference when protein and albumin were reduced in their levels in the serum of patients with renal failure. Otherwise, serum albumin concentration is significantly lower in renal failure patients when compared  with those of the control group.

A reduction in the rate of albumin synthesis which may be caused by metabolic acidosis, impaired protein intake, and inflammation show a significant decrease in albumin level in HD patients [35,( Carrero, J.J., Stenvinkel, P., Cuppari, L., Ikizler, T.A., Kalantar-Zadeh, K., Kaysen, G., Mitch, W.E., Price, S.R., Wanner, C., Wang, A.Y., ter Wee, P., Franch, H.A. (2013)23(2): 77– 90) Changes in the structure of basement membrane of glomeruli which consequent lead to the leakage of albumin and some low molecular weight proteins. Proteinuria is considered as a marker of renal disease progression (Pandian J, Amit Kumar K, Swaminathan A.2017).

Restriction of protein intake (Sathiyanarayanan, S., Shankar M.P. and Prabhakar, E.R. (2013))and protein malnutrition may attribute to such decrease in albumin and total protein of the serum of the corresponding patients Afshar, R., Sanavi, S., Izadi-Khah, A. (2007). This result is similar to the previous studies done by (Abdul Wahid, H. Kismat, M.T. and Asma, Y. 2006:48(4): 431-34) ,Lefta, A. (2009)], they suggested that proinflammatory cytokines (TNF-a and interleukins) induce an acute phase response decreases hepatic synthesis of albumin and increases its catabolism the degradation of albumin. Thus, much of the observed association of albumin level with outcomes may be attributed to inflammation rather than malnutrition in the ESRD population (Kaysen, G.A.2001:1549-1557)

**3.6-Impact of dialysis on uria & uric acid :**

According to our findings the urea and uric acid showed a significant decrease in post dialysis with (56.89±5.87 and 7.458±0.532) respectively. Serum creatinine showed sharp decrease after dialysis

The occurrence of balanced amount of consumed proteins is also an important step to avoid excessive production of urea (Gonella GM.2016;3: 2454- 7379) The hemodialytic process carried out in the studied patients was perceived to be efficient, because significantly reduced levels of creatinine and urea were recorded ensuingly. According to Draczevski and Teixeira (Hassen, Hussein F., et al.2018, pp. 50-57.), the assessed pre- and post-hemodialysis urea levels, obtained reflected a significant reduction in serum levels, indicating hemodialysis as an efficient technique. Removal of waste during dialysis also depends upon proper timings of dialysis, patient awareness, and appropriate dialyzer and dietary habits of patients (Merzah KS, Hasson SF.2015;4:75-79) Urea and creatinine levels are important biomarkers as they play a pivotal role in diagnosis and follow-up of kidney failure. Urea, one of the by-products of protein metabolism, accumulates in the blood of patients with kidney failure and causes uremia (Entedhar RS, Nawal AM2016;5:190-195),(Shivananda Nayak2002;98-99)

Table (3) Show liver function, Lipid profile, and kidney markers complications in chronic kidney frailer patients.

| Parameters | Patient | | P-Value |
|---|---|---|---|
| | Before | After | |
| Cholesterol (U/L) | 173.7±22.5 | 215.8±21.84 | ↑ 0.001 |
| | | | |
| Triglyceride | 258.5(127.5-440) | 282.5(145.5-778.8) | NS |
| HDL | 38.77±5.525 | 49.2±7.438 | ↑ 0.001 |
| LDL | 73.9±13.4 | 108.3±17.73 | ↑ 0.01 |
| UREA | 151.8±11.9 | 56.89±5.87 | ↓ 0.001 |
| CREATININ | 10.18±0.841 | 4.311±0.408 | ↓ 0.001 |
| URIC ACID | 7.458±0.532 | 2.589±0.238 | ↓ 0.001 |
| ALBUMIN | 4.75(4.575-5.025) | 5.5(4.6-565) | NS |
| GOT (U/L) | 17.1±1.997 | 23.2±1.67 | ↑ 0.001 |
| GPT (U/L) | 13.4±2.4 | 16.1±2.387 | ↑ 0.001 |
| ALP (U/L) | 121.8±21.27 | 145.9±23.7 | ↑ 0.001 |
| TBIL (mg/dl) | 0.4(0.4-0.525) | 0.55(0.375-0.7) | NS |

**Conclusion:**

A firm relationship is observed between serum creatinine and serum urea levels among renal failure patients.hemodialysis forms an effective process as an efficient and indispensible process for the filtration of undesired metabolites such as creatinine and urea and electrolytes at a considerable range, hence decreasing burden over kidneys. Therefore, it is recommended that all patients be screened appropriately before and after dialysis to avoid complications.

**Recommendations:**

Depending on our study we recommended for the further studies to

1-To evaluate and study Erythropoietin level in chronic kidney patients
2-to measure Hepcidin level in the Blood

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