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# Comparison of Hepcidin Level, Blood Parameters and Inflammatory Markers in High Flux Hemodialysis and Hemodiafiltration Patients

**M. Y. Naji Al Atbee (MD)[*1], H. Sami Tuama (MD)[2], R. Abdulwahid Gatee (MD)[3], M. M. Salih Hasan (MD)[3], S. Ali Salman (MD)[4], Gh. Hasan Mahmood (Pharm D, PhD)[5]**

1.College of Medicine, University of Basrah, Basra, Iraq.
2.Family Physician, Basrah Primary Health Care, Basra, Iraq.
3.Nephrology and Dialysis Center, Basrah Teaching Hospital, Basra, Iraq.
4.College of Science, Tikrit University, Tikrit, Iraq.
5.College of Pharmacy, Al-Farahidi University, Baghdad, Iraq.

•**Corresponding Author: M. Y. Naji Al Atbee (MD)**
**Address:** College of Medicine, University of Basrah, Basra, Iraq..
**Tel:** +964 (1) 8868520. **E-mail:** goz.mr@yahoo.com

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## ABSTRACT

**Background and Objective:** Healthy kidneys filter wastes of various molecular masses (up to 40-60 kDa). Traditional hemodialysis only removes low-molecular-weight molecules (500 Da), not middle-weight substances (>500 Da). Therefore, most of these compounds remain as expected uremic toxins. Therefore, this study aims to determine the association of Hepcidin and anemia with inflammatory markers in dialysis cases on high flux hemodialysis (HD) compared with hemodiafiltration (HDF).

**Methods:** This cross-sectional study was conducted on 203 patients in the age range of 20-70 years old with end-stage kidney disease who were undergoing hemodialysis using high flux and hemodiafiltration methods. Venous blood samples were taken without anticoagulation and ferritin, CRP and hepcidin levels were analyzed and compared.

**Findings:** This study involved 203 cases with end-stage renal disease, with 103 in the HD group and 100 in the HDF group. The mean age of the cases was 49.5±15.5 years. Among the patients in the HD group, 79.9% had hypertension, while only 23% of the patients in the HDF group had hypertension. Additionally, 35.9% of the cases in the HD group had diabetes mellitus (DM), while 75% of the cases in the HDF group had DM. There were significant differences between the mean number of white blood cells (8.27±7.95 vs. 6.20±4.14), CRP (13.88±17.45 vs. 6.48±10.40) and hepcidin (2981.47±2325.38 vs. 1010.45±1136.18) between the groups ($p<0.05$).

**Conclusion:** The results of this study showed that individuals receiving HDF have low-grade inflammation, less anemia, and a superior response to erythropoietin. Therefore, for patients with elevated inflammatory markers, the HDF dialysis modality is suggested.

**Keywords:** *Hepcidin, Anemia, Inflammatory Markers in Dialysis, High Flux Hemodialysis (HD), Hemodiafiltration (HDF).*

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## Introduction

Human kidneys can filter wastes with a broad range of molecular masses when they are functioning at their best (up to 40-60 kDa). On the other hand, middle-molecular-weight compounds (>500 Da) cannot be successfully eliminated by traditional hemodialysis (HD), and only low-molecular-weight molecules (500 Da) can. These materials, the majority of which are believed to be uremic toxins (1), may do this and increase the uremic burden. Based on research findings, these individuals have high levels of uremic toxins in their bodies, which in some way contribute to the many adverse effects of uremia, including significant cardiovascular disease (1-3).

To help with the removal of medium-sized molecules, a membrane with a high permeability were created (4). This enables greater hemofiltration and, as a consequence, improves solute removal by convection. Hemodiafiltration (HDF) was first launched in 1975. This procedure increased the clearance of molecules of intermediate molecular weight by integrating the convective and diffusive clearances of HD and hemofiltration (5). The introduction of HD machines that conducted HDF with on-line synthesis of replacement fluid removed a hurdle to this technique: the need for vast volumes of replacement solution (6). It was hoped that HDF would provide a more powerful therapy that might lower the high mortality rate of the uremic population since this kind of dialysis has been shown to be clinically safe. However, a Cochrane meta-analysis found insufficient evidence to suggest HDF's advantage over traditional HD (7). In the same study, it was reported that neither participants nor researchers were blinded to the intervention in any randomized experiment comparing HD with HDF.

The body's main iron regulator is hepcidin, a 25-amino-acid peptide hormone generated via the liver. It controls intestinal iron absorption and hepatic and reticuloendothelial iron release to maintain iron homeostasis. Iron and inflammation affect serum hepcidin. Hepcidin may be a better indicator of hemoglobin (Hb) synthesis capability than current iron measurements, hence its role in iron homeostasis has garnered interest. This hormone can treat iron problems. ESRD patients have not been examined for its role in erythropoiesis. Incident dialysis patients' clinical factors impacting hepcidin levels are unknown. Cross-sectional studies show that frequent peritoneal dialysis (PD) patients had lower serum hepcidin and pro-hepcidin than HD patients. PD patients also had greater serum Hb and respond better to ESAs than HD patients. Thus, serum hepcidin levels may be good indications of iron control in anemia treatment and ESA response in incident dialysis patients, especially by modality. Inflammation is part of the complicated physiologic response to infection, tissue injury, ischemia, and autoimmune disorders (8).

Acute-phase response indicators such C-reactive protein (CRP, >0.3 mg/dL) and pro-inflammatory cytokines enhance CRP production. The toxic uremic environment and dialysis produce CKD inflammation. Inflammation directly affects most iron indicators, including ferritin and hepcidin, making their interpretation difficult (9). Inflammation-induced hepcidin increases trap iron in macrophages and hepatocytes, causing FID (10). In CKD patients, inflammation is associated with FID Anemia (FIDA) (11-13), necessitating larger IV iron doses to meet Hb goals. Inflammation increases ferritin and hepcidin, making HD patients hyporesponsive to iron treatment and ESA. In contrast, intensive intravenous iron treatment (IIT) may increase inflammation in ESRD patients, disrupting iron metabolism (14). Inflammation affects ferritin, hepcidin, and IDA treatment in CKD patients. This study aims to determine the association of Hepcidin and anemia with inflammatory markers in dialysis patients on high flux hemodialysis in comparison with hemodiafiltration.

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## Methods

In this cross-sectional study, samples were collected at the Basrah Teaching Hospital's nephrology and dialysis department from August 2022 to January 2023 with the ethical code BU-2022-1-121. All participants in the study provided written informed consent. The research included 203 individuals in the age range of 20 and 70 who had end-stage renal disease undergoing dialysis. The diagnosis of ESRD was made if patient's Glomerular Filtration Rate (GFR) was below 15 mL/min/1.69 $m^2$ (15).

For each patient, 5 mL of venous blood was collected using disposable syringes and placed in plain tubes without anticoagulant. The tubes were labeled with the patient's name and the collection date. The samples were handled immediately. From the collected blood samples, serum ferritin, complete blood count, and C-reactive protein (CRP) were isolated from the blood serum using a centrifuge at 10,000 rpm for 5 minutes at 4°C. The concentration of serum hepcidin was determined using the competitive enzyme-linked immunosorbent assay as mentioned previously. In summary, 96-well plates were coated with goat anti-rabbit IgG (Fc) antibody and incubated overnight at 4°C. Then, the plates were blocked with bovine serum albumin for two hours at room temperature. Then, they were incubated with a privately generated rabbit anti-human hepcidin antibody for two hours at room temperature. The wells were pipetted with reference samples, standards, and study samples (20-fold diluted). The plates were cleaned and exposed to the o-phenylenediamine substrate for 15 minutes in the dark, following incubation with a streptavidin-peroxidase conjugate for an hour at room temperature.

In our research, ferritin and C-reactive protein were used as inflammatory indicators. Hemodialysis was performed at our facility using high flux B Braun hemofilters, with an average treatment duration of 4 hours to achieve an online Kt/V (dialysis adequacy) level of 1.3. Hemodiafiltration was also conducted at our facility using a high flux hemofilter with a convective volume exceeding 20 L and an average operating time of 4 hours.

For data analysis, SPSS V.20 software was used. Mean values and standard deviations (SD) were calculated to characterize the research population. The T-test was employed to evaluate the statistical significance of data differences, considering a significance level of $p<0.05$.

## Results

In this cross-sectional study, a total of 203 cases participated. The patients had a mean age of 49.5±15.5 years. Among the patients undergoing HD sessions, 79.9% had hypertension, whereas only 23% of patients receiving HDF had hypertension. Additionally, 35.9% of cases in the HD group had diabetes mellitus (DM), while 75% of cases in the HDF group had DM (Table 1).

**Table 1. Distribution of patients in both groups if they have hypertension or DM**

| Variables | Session type | | |
|---|---|---|---|
| | HD Number(%) | HDF Number(%) | Total Number(%) |
| **Hypertension** | | | |
| No | 30(29.1) | 77(77) | 107(52.7) |
| Yes | 73(70.9) | 23(23) | 96(47.3) |
| Total | 103(100) | 100(100) | 203(100) |
| **Diabetes Mellitus** | | | |
| No | 66(64.1) | 25(25) | 91(44.8) |
| Yes | 37(35.9) | 75(75) | 112(55.2) |
| Total | 103(100) | 100(100) | 203(100) |

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As illustrated in Figure 1, the distribution of patients based on chronic diseases in both groups is as follows: 83 patients undergoing HD sessions did not have any chronic diseases, while 77 patients receiving HDF also did not have any chronic diseases. Additionally, 12 patients in the HD group had heart failure, compared to 10 patients in the HDF group. All of the chronic diseases depicted in Figure 1 were observed in both groups. As depicted in figure 2, a significant negative correlation between Hepcidin and C-reactive protein (CRP) was observed (p=0.037).

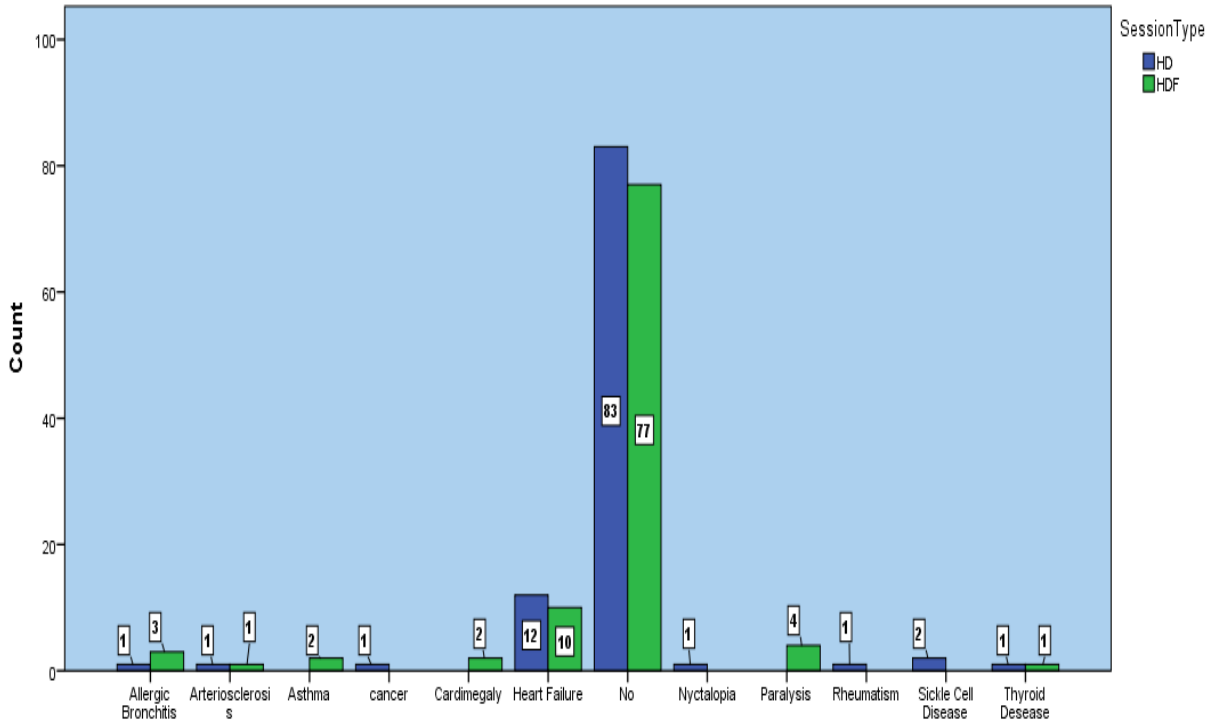



**Figure 1. Cases based on chronic disease in both groups**

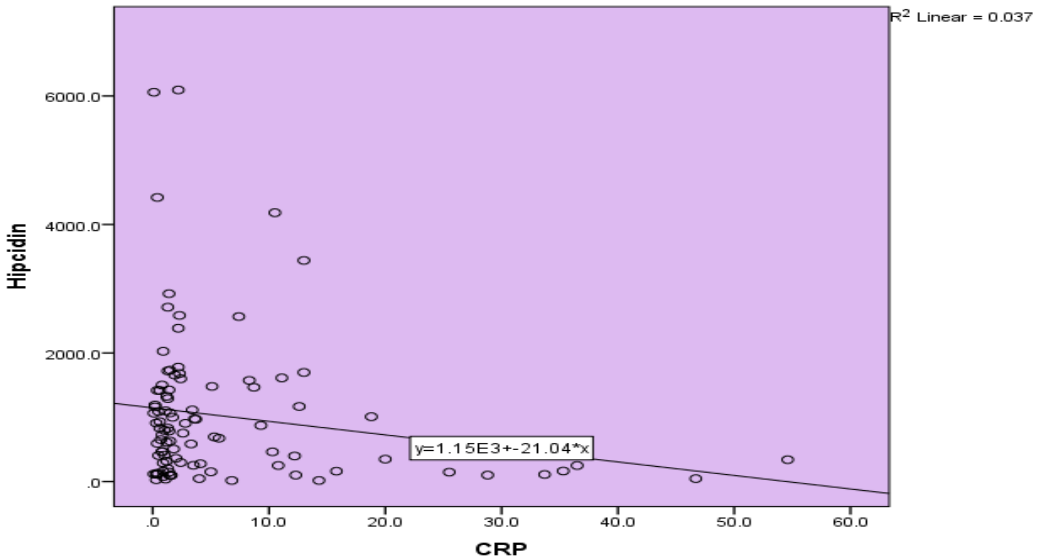



**Figure 2. correlation between Hepcidin and CRP**

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According to Table 2, significant differences were observed in the mean values of WBC, CRP, and Hepcidin between both groups ($p<0.05$). Patients undergoing HD had higher mean values of WBC, CRP, and Hepcidin compared to patients undergoing HDF. However, no significant differences were found in the mean values of age, HB, and ferritin between the two groups.

As presented in table 3, the levels of Hb, RBC, and HCT factors and plasma levels of the cytokines between NA and anemic (A) cases were investigated. Compared to NA cases, the Hb levels mainly reduced in (A) cases. Also, the rate of anemia and inflammatory cytokines correlations were presented for three cases None Anemia (NA), Moderate Anemia (MN) and Intensive Anemia (IA). The average plasma cytokine vs the anemia varying degree were presented in table 4.

**Table 2. Differences between mean of age, HB, ferritin, WBC, CRP and Hepcidin according to both groups**

| Variables and type | Number | Mean±SD | p-value |
|---|---|---|---|
| **Age** | | | |
| HD | 103 | 49.43±15.98 | 0.9 |
| HDF | 100 | 49.66±15.18 | |
| **Hb** | | | |
| HD | 103 | 8.89±1.67 | 0.8 |
| HDF | 100 | 8.96±1.60 | |
| **Ferritin** | | | |
| HD | 103 | 310.39±354.15 | 0.18 |
| HDF | 99 | 248.46±301.40 | |
| **WBC*** | | | |
| HD | 103 | 8.27±7.95 | 0.021 |
| HDF | 100 | 6.20±4.14 | |
| **CRP**** | | | |
| HD | 103 | 13.88±17.45 | 0.0001 |
| HDF | 100 | 6.48±10.40 | |
| **Hepcidin** | | | |
| HD | 103 | 2981.47±2325.38 | 0.0001 |
| HDF | 100 | 1010.45±1136.18 | |

***White Blood Cells, ****C-reactive protein**

**Table 3. Variation of the hematological factors and inflammatory cytokine in anemic and nonanemic**

| Parameter | NA Mean±SD | A Mean±SD | p-value |
|---|---|---|---|
| Hb | 13.2±1.5 | 8.8±2.3 | 0.003 |
| RBC | 5.01±0.77 | 4.58±2.9 | 0.001 |
| HCT | 42.01±9.98 | 32.88±9.8 | 0.001 |

**Table 4. Association of anemia and inflammatory cytokines**

| Parameter | NA Mean±SD | MA Mean±SD | IA Mean±SD |
|---|---|---|---|
| Hb (g/dl) | 12.9±1.2 | 9.9±0.6 | 5.2±0.3 |

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## Discussion

In this study, a significant negative correlation was found that between CRP and Hepcidin level in patients undergoing HD and HDF. Also, patients receiving HD had significantly higher levels of WBC, CRP and Hepcidin in compare with HDF. Moreover, it was found that there is a significant association between anemia and inflammatory cytokines.

This is comparable to a study that found that at day 60, post-dialysis blood hepcidin was about 40% less than the HDF cases in the HD ones. Hepcidin is removed by both low- and high-flux HD and has a molecular mass of 2,791 Da (14). The mean base level of serum hepcidin determined by mass spectrometry is 4.3 nM (in 0.6-14) (16, 17). In studied cases, blood hepcidin levels were consistently within these ranges following HDF treatment (mean 7, in 2.5-13), although 30% of cases had serum hepcidin levels above these ranges (mean 11, in 2.5-19). In current research, there was no significant difference in the Hb between HD and HDF groups. Previous studies have also shown that patients treated with HDF had higher hemoglobin levels and needed less erythropoietin, according to certain findings (18-20).

An earlier prospective study (21) compared patients who were converted to HDF with those who continued on HD treatment and found that Protein Catabolic Rate (PCR) was significantly lower in the HDF group at 6 and 12 months compared to the HD medication group. A different study, however, found no appreciable difference between PCR levels after six months of HD and PCR levels after six months of HDF (22). When patients were switched to OL-HDF, we also observed a decrease in PCR levels, albeit the difference was not statistically significant. Remaining renal function affects PCR, and errors are mainly dependent on the Kt/V used to calculate PCR (23). The findings show that OL-HDF has no harmful effects on patients' nutritional status; nonetheless, in these cases, further investigation of dietary situations using other techniques would be necessary. Contrary to prior studies that claimed there was no association between hepcidin blood levels and IL-6 ($p=0.582$) or CRP ($p=0.783$) levels of inflammation, the present investigation found a substantial negative correlation between Hepcidin and C-reactive protein (24).

The mean WBC levels of patients with HD were greater than those with HDF, however there was no significant difference in ferritin levels between the two cases. In comparison to HD, HDF therapy was linked to a tendency towards higher MCV values and lower ferritin levels at day 60 ($p=0.08$ for both). No more notable modifications were seen across all dialysis methods (24). Pyuria was shown by the authors to be an unreliable indication of UTIs in HD patients (25). There has recently been a link established between Urinary Tract Infection (UTI) and pyuria threshold levels (greater than 5, 10, 50, and 100 WBC/HPF). When the cutoff value was increased, pyuria's specificity increased but its sensitivity decreased. As a diagnostic tool for UTI, several pyuria cutoffs were shown to be insufficient in terms of sensitivity and specificity. Additionally, Dipstick urinalysis to detect the presence of nitrites had a high specificity (95%) but a poor sensitivity (15-21%) (26). When Nadeem et al. examined urine samples from 90 HD patients, they discovered that pyuria had a 100 percent sensitivity rate and a 61.8% specificity rate for UTIs. The authors suggested that samples with positive pyuria should be cultured to confirm UTI (27) given the low specificity and high positive predictive values, which were 35.5% and 100%, respectively.

Hepcidin has an inverse relationship with C-reactive protein, and the mean white blood cell count, C-reactive protein, and Hepcidin levels of HD patients are all higher than those of HDF patients. As opposed to hemodialysis patients, individuals receiving HDF have low-grade inflammation, less anemia, and a superior response to erythropoietin. For patients with elevated inflammatory markers, the HDF dialysis modality is suggested.

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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