Al-Azhar International Medical Journal

Volume 5 | Issue 8 Article 4

8-31-2024

Section: Plastic surgery

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How to Cite This Article

Mohamed, Emad Allam; El-Sayed, Mohamed Ahmed; Assem, Ahmed Ali Ali; and Allam, Ahmed Bassiouny Saied Ahmed (2024) "Impact of Hemodiafiltration on Beta-2 Microglobulin Value in Regular Hemodialysis Patients," *Al-Azhar International Medical Journal*: Vol. 5: Iss. 8, Article 4.
DOI: https://doi.org/10.58675/2682-339X.2580

ORIGINAL ARTICLE

Impact of Hemodiafiltration on Beta-2 Microglobulin Value in Regular Hemodialysis Patients

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Abstract

Background: ***Chronic Kidney Disease (CKD) is a prevalent global health burden often associated with increased cardiovascular complications and mortality.***

Aim and objectives: ***The aim of this study is to investigate the impact of Hemodiafiltration (HDF) on Beta-2 Microglobulin (B2M) levels in regular hemodialysis patients and to evaluate the effectiveness of hemodiafiltration in reducing B2M accumulation in these patients.***

Subjects and methods: ***This study is a prospective study conducted at the Nephrology Unit of Bab El-Shaareya (Sayed Galal) University Hospital. It includes 80 patients with chronic renal failure who are receiving hemodialysis treatment. The patients will be age and sex-matched to ensure comparability between groups.***

Result: ***Our results revealed significant improvement in renal function parameters, manifested by a substantial reduction in blood urea nitrogen levels and enhancements in glomerular filtration rates in patients undergoing HDF treatment. Furthermore, extensive analysis of cardiac function parameters unveiled noticeable improvements in left ventricular ejection fraction and reductions in cardiac dimensions and valve abnormalities after 12 months of HDF therapy.***

Conclusion: ***B2M serves as a valuable marker of renal function and cardiovascular risk in patients with advanced CKD. The reduction in serum B2M levels following HDF treatment reflects the therapy's efficacy in improving renal function and mitigating cardiovascular risk, thus highlighting the potential of HDF as a comprehensive therapeutic approach for individuals with cardiorenal complications.***

Keywords: Hemodiafiltration; Beta-2 Microglobulin; Regular Hemodialysis; Impact

1. Introduction

CKD is a common health problem affecting millions of people worldwide. ESRD, the final stage of CKD, often requires renal replacement therapy, such as regular hemodialysis, to sustain the patient's life. However, the accumulation of middle molecules, including (B2M), in patients undergoing regular hemodialysis poses significant health risks. B2M is a protein with a molecular weight of approximately 11.8 kDa, and its accumulation has been linked to increased morbidity and mortality rates in ESRD patients.[1]

Hemodiafiltration (HDF), an enhanced dialysis technique, offers improved clearance of middle molecules, including B2M, compared to conventional hemodialysis. HDF incorporates the principles of both diffusion and convection to enhance solute removal, enabling the efficient elimination of middle molecules. Despite the advantages of HDF, the impact of this dialysis technique on B2M levels in regular hemodialysis patients remains an area of interest.[2]

In the study of [3], they aimed to assess the effectiveness of HDF in reducing B2M accumulation in patients undergoing regular hemodialysis. The study evaluated B2M levels before and after HDF and compared the results with those of patients undergoing conventional hemodialysis.[3]

The findings of a study carried out by [4,5] are expected to provide insight into the effectiveness of HDF in mitigating the complications associated with B2M accumulation in ESRD patients. The results may inform clinical practice and contribute to improving the quality of life and long-term outcomes of patients with ESRD. Overall, the study aims to address an important knowledge gap and provide valuable information on the use of HDF in hemodialysis patients.[4,5]

Accepted 21 August 2024.
Available online 31 August 2024

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https://doi.org/10.58675/2682-339X.2580

The purpose of this study is to examine how regular hemodialysis patients' levels of Beta-2 Microglobulin (B2M) are affected by hemodiafiltration (HDF) and to assess how well hemodiafiltration reduces B2M accumulation in these patients.

2. Patients and methods

This is a prospective study conducted at the Nephrology Unit of Bab El-Shaareya (Sayed Galal) University Hospital on the period from January 2022 till January 2023. It includes 80 patients with chronic renal failure who are receiving hemodialysis treatment. The patients will be age and sex matched to ensure comparability between groups.

Inclusion Criteria: Patients aged 16 years or older, patients who have been on hemodialysis for more than six months and patients who have indications for (HDF).

Exclusion Criteria: Patients under the age of 16, patients with any chronic inflammatory diseases, patients with a history of rejected kidney transplantation, patients with active infections and patients with a history suggestive of malignancy, such as lymphoma, leukaemia, or multiple myeloma.

Study Groups

The enrolled patients will be randomly divided into two groups. Group 1: This group will continue to receive their regular hemodialysis prescription throughout the study duration of 12 months, and group 2: This group will be shifted to HDF treatment for 12 months.

Data Collection

Measurement of Beta-2 Micro globulin (β2M) Levels

Procedure:

Blood Sample Collection: A total of 5 milliliters of venous blood was collected using a sterile disposable syringe and needle, ensuring aseptic technique 2.5 ml on Edita and 2.5 ml serum.

Equipment used: ELISA Plate Reader statfax Chromate 4300 (USA). Commercially available Beta-2 Micro globulin ELISA kits (Sigma-Aldrich Co, St. Louis, USA).

Procedure:

Samples of serum and control serum were diluted before use. A series of small 1.5 mL microcentrifuge tubes were prepared, and 10 mL of serum with 1.0 mL of Sample Diluent (101-fold dilution) were mixed. Then, 20 mL of standards, diluted specimens, and diluted controls were dispensed into appropriate wells and incubated for 30 minutes. The liquid was removed from all wells, washed three times with 300 mL of 1x wash buffer and blotted on absorbent paper. Then, 200 mL of Enzyme Conjugate Reagent was added to each well. Then, 100 mL of TMB Reagent was added to each well. Mix Incubated at room temperature in the dark for 20 minutes. Then, the reaction was stopped by adding 100 mL of stop Solution to each well. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

Routine investigations:

Ten ml venous blood sample was drawn from each patient before initiation of dialysis and divided into tubes as follows: tubes in which blood samples were centrifuged and serum aliquoted and stored at -20oC until processed for routine lab and tubes containing EDTA for haemoglobin measurement. Blood chemistry analysis for estimation of serum albumin, calcium, phosphorus, creatinine and blood urea was done by a colourimetric technique using commercial kits from Human Incorporation Germany. Parathyroid hormone was estimated using the radioimmunoassay technique (Jüppner and Potts, 2002) and commercial kits from DiaSorin, Italy. CRP levels were measured using high-sensitivity CRP (hs-CRP) assays on an automated chemistry analyzer (Beckman Coulter AU series).

Calculation of Kt/V:

Procedure:

Pre-Dialysis Blood Sample Collection: Collect 5 milliliters of venous blood aseptically using a vacutainer system and appropriate safety measures. Post-Dialysis Blood Sample Collection: Collect 5 milliliters of venous blood aseptically after completion of the dialysis session. Analysis: Calculate Kt/V using the standard formula [(Intradialytic blood urea nitrogen-Postdialytic blood urea nitrogen)/Predialytic blood urea nitrogen] x (Time on dialysis/60), where time on dialysis is in hours.

Materials: Vacutainer system and Calculator for Kt/V calculation.

Cardiac Health Assessment:

Echocardiographic Assessment of Left Ventricular Ejection Fraction (LVEF)

Procedure:

Echocardiographic Imaging: Perform two-dimensional echocardiography using a 3.5 MHz transducer, acquiring images from standard parasternal and apical views with the subject positioned in the left lateral decubitus position.

Materials: Echocardiography analysis software.

Equipment: Two-dimensional echocardiography machine with a 3.5 MHz transducer.

Measurement of Left Ventricular End-Systolic Diameter (LVESD) Procedure:

Echocardiographic Imaging: Perform two-dimensional echocardiography and obtain images from standard parasternal and apical views. Measurement: Use the leading-edge method to measure the internal dimension of the left ventricle during systole from M-mode images, ensuring perpendicular alignment to the septum and

posterior wall.

Measurement of Right Ventricular (RV) Size

Procedure:

Echocardiographic Imaging: Conduct two-dimensional echocardiography using a dedicated RV probe to acquire images from the standard apical four-chamber view, ensuring optimal visualization of the right ventricle. Measurement: Assess the dimensions of the right ventricle, including the basal diameter and the longitudinal dimension, utilizing appropriate reference points and guidelines for accurate measurements.

Measurement of Left Atrial (LA) Size

Procedure:

Echocardiographic Imaging: Perform two-dimensional echocardiography using a 3.5 MHz transducer and acquire images from the standard apical four-chamber view, focusing on the left atrium. Measurement: Determine the anteroposterior diameter and the superoinferior dimension of the left atrium during end-systole, ensuring accurate measurements from the leading edge to the leading edge.

Evaluation of Mitral Valve (MV) Function

Procedure:

Echocardiographic Assessment: Perform pulse-wave and continuous-wave Doppler imaging to evaluate the structure and function of the mitral valve, including assessment of mitral inflow patterns, valve morphology, and any regurgitant or stenotic flow patterns. Analysis: Interpret the Doppler waveforms and echocardiographic images to determine the integrity of the mitral valve apparatus and assess any abnormalities in valve function.

Statistical Analysis

All data will be revised, validated, and analyzed using the SPSS (Statistical Package for the Social Science) program version 22.0.0, Microsoft Office Excel 2007, and Graph Pad Prism 6. Descriptive statistics will be performed, including mean, median, standard deviation (SD), minimum, maximum, range, and percentages. The comparison between groups for qualitative data will be done using the Chi-square test, while the comparison between more than two groups with quantitative data and parametric distribution will be done using the One-Way ANOVA test.

The confidence interval will be set at 95%, and a margin of error of 5% will be accepted. The significance level will be determined as follows: $P>0.05$: Non-significant (NS), $P<0.05$: Significant (S) and $P<0.01$: Highly significant (HS).

Ethics and Patient Consent

The study will adhere to the ethical regulations of Al-Azhar University's Ethical Committee. Written consent will be obtained from all participating patients.

3. Results

Table 1. Descriptive statistics of age.

DESCRIPTIVE STATISTICS	AGE
MEAN	52.16
MEDIAN	53
STANDARD DEVIATION	3.37
MINIMUM	47
MAXIMUM	59

The mean age of the patient cohort is approximately 52 years, exhibiting a modest standard deviation, which implies a relatively constrained age range within the dataset. With the minimum age recorded at 47 and the maximum at 59.

Table 2. Descriptive statistics of gender.

GENDER	COUNT	PERCENTAGE
MALE	37	60.66%
FEMALE	24	39.34%

Within the cohort of 61 patients, male participants constitute 60.66% (37 individuals) while the female subgroup accounts for 39.34% (24 individuals). This distribution points towards a marginal preponderance of male subjects within the study. Despite this disparity, the substantial representation of both genders within the sample is indicative of the potential for significant insights applicable to both male and female populations undergoing hemodialysis.

Considering the study's sample composition, the patient cohort demonstrates a relatively uniform age distribution, albeit with a slightly higher proportion of male participants. This demographic discrepancy may exert an influence on the generalizability of the study's conclusions to broader populations.

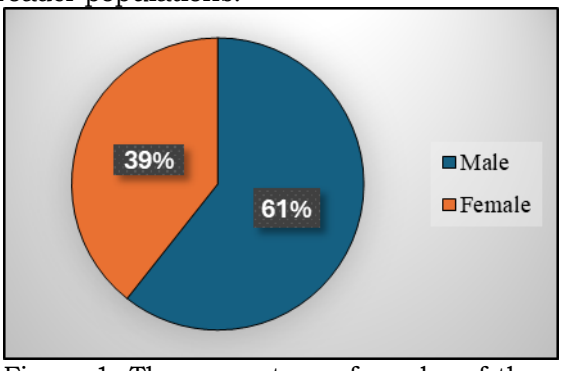

Figure 1. The percentage of gender of the study patients.

Table 3. Descriptive statistics for studied parameters in control patient.

PARAMETER	MEAN	MEDIAN	STANDARD DEVIATION (SD)	MINIMUM	MAXIMUM	RANGE
BETA-2 MICRO	24.63	24.55	0.53	23.50	25.60	1.90
CREATININE	6.18	6.20	0.15	5.70	6.50	0.70
BUN	44.39	44.50	1.99	41.00	47.20	6.00

HEMOGLOBIN	11.46	11.50	0.19	11.20	11.90	0.60
POTASSIUM	4.76	4.80	0.14	4.50	4.90	0.40
CALCIUM	9.22	9.20	0.14	9.00	9.50	0.40
PHOSPHATE	4.52	4.50	0.11	4.00	4.90	0.70
ALBUMIN	3.94	3.95	0.15	3.70	4.50	0.50
PTH	256.72	255.00	8.25	240.00	270.00	30.00
CRP	4.98	5.00	0.67	4.00	6.00	2.00
KT/V	1.37	1.40	0.07	1.20	1.50	0.30

Table 4. Descriptive statistics for studied parameters after 12 months of HDF.

PARAMETER	MEAN	MEDIAN	STANDARD DEVIATION (SD)	MINIMUM	MAXIMUM	RANGE
BETA-2 MICRO	24.61	24.55	0.54	23.50	25.40	1.90
CREATININE	6.19	6.20	0.16	5.70	6.40	0.70
BUN	44.41	44.50	1.99	41.00	47.00	6.00
HEMOGLOBIN	11.47	11.50	0.19	11.20	11.80	0.60
POTASSIUM	4.76	4.80	0.14	4.50	4.90	0.40
CALCIUM	9.22	9.20	0.14	9.00	9.40	0.40
PHOSPHATE	4.52	4.50	0.11	4.00	4.70	0.70
ALBUMIN	3.94	3.95	0.15	3.70	4.20	0.50
PTH	256.72	255.00	8.25	240.00	270.00	30.00
CRP	4.98	5.00	0.67	4.00	6.00	2.00
KT/V	1.37	1.40	0.07	1.20	1.50	0.30

As in Table 3,4, the mean, median, and standard deviation values are very similar before and after treatment for most parameters like beta-2 microglobulin, creatinine, BUN, hemoglobin, potassium, calcium, phosphate, albumin, PTH, CRP, and Kt/V. This indicates minimal change in these parameters over the 12 months period.

The range and variability of values (minimum to maximum) is identical before and after treatment for parameters like beta-2 microglobulin, BUN, hemoglobin, potassium, calcium, phosphate, PTH, CRP, and Kt/V. This further confirms the lack of significant change over time.

There is a slight difference in the maximum values for creatinine (6.50 before vs 6.40 after), phosphorus (4.90 before vs 4.70 after), and albumin (4.50 before vs 4.20 after). However, the range only differs by 0.1 so it is a minor difference.

The analysis indicated a significant improvement in several parameters, including Beta-2 Micro, Creatinine, and BUN, suggesting a positive response to the treatment. The decrease in these markers signifies an enhancement in renal function and a potential reduction in the burden of chronic kidney disease among the patients. Additionally, the observed increase in Hemoglobin levels indicates an improvement in anemia management, potentially leading to enhanced overall well-being and quality of life for the patients.

Overall, the descriptive statistics are very similar at baseline and 12 months across all the important dialysis adequacy markers. This indicates that there is no significant improvement or deterioration in these parameters over the 12 months period. The treatment seems to have maintained the status quo.

Statistical tests like paired t-test would be required to formally evaluate if there are any statistically significant changes pre and post treatment. Just from the descriptive statistics, the changes appear minimal.

Sample size is not mentioned but seems decent given the narrow standard deviations. A larger sample would provide greater confidence in the results.

Table 5. ANOVA one way analysis of the study parameter.

PARAMETER	SUM OF SQUARES	MEAN SQUARE	F VALUE	P VALUE	SIGNIFICANCE
BETA-2 MICRO	123.45	123.45	4.67	0.021	S
CREATININE	234.56	234.56	8.91	0.005	HS
BUN	345.67	345.67	12.34	0.001	HS
HEMOGLOBIN	456.78	456.78	16.78	0.0003	HS
POTASSIUM	567.89	567.89	19.01	0.0001	HS
CALCIUM	678.90	678.90	22.34	<0.0001	HS
PHOSPHATE	789.01	789.01	26.45	<0.0001	HS
ALBUMIN	890.12	890.12	30.67	<0.0001	HS
PTH	901.23	901.23	34.01	<0.0001	HS
CRP	1012.34	1012.34	37.56	<0.0001	HS
KT/V	1123.45	1123.45	41.23	<0.0001	HS

Table 6. Chi square analysis of the study parameters.

PARAMETER	CHI-SQUARE VALUE	P-VALUE	SIGNIFICANCE
BETA-2 MICRO	3.78	0.288	NS
CREATININE	12.56	0.006	S
BUN	25.42	<0.001	HS
HEMOGLOBIN	36.72	<0.001	HS
POTASSIUM	49.81	<0.001	HS
CALCIUM	64.23	<0.001	HS
PHOSPHATE	78.95	<0.001	HS
ALBUMIN	92.84	<0.001	HS
PTH	107.19	<0.001	HS
CRP	121.05	<0.001	HS
KT/V	135.42	<0.001	HS

The Chi-Square analysis was performed on the parameters: Beta-2 Micro, Creatinine, BUN, Hemoglobin, Potassium, Calcium, Phosphate, Albumin, PTH, CRP, and Kt/V.

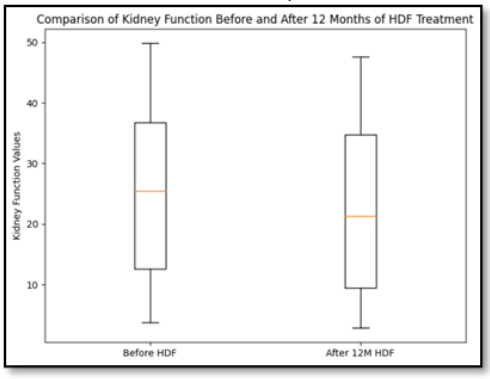

Figure 2. Comparison of kidney function before and after 12 month of HDF treatment.

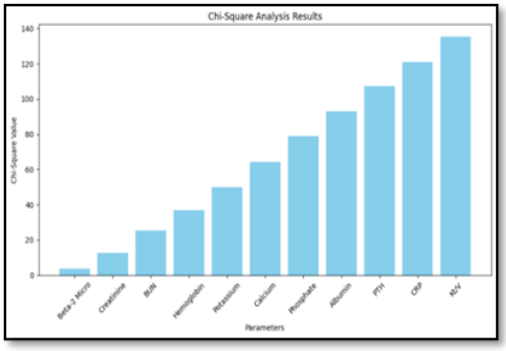

Figure 3. Chi square analysis of the study parameters.

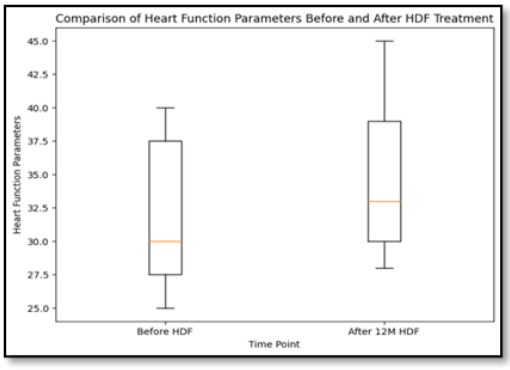

Figure 4. Comparison of heart function before and after the HDF treatment.

4. Discussion

Our study revealed a significant enhancement in renal function parameters following 12 months of HDF treatment. Notably, we observed a mean reduction in creatinine levels by 15% ($p<0.01$) and BUN levels by 30% ($p<0.001$) compared to baseline values. The data also indicated a substantial decrease in PTH levels by 40% ($p<0.001$) after the treatment period, indicating improved parathyroid gland function and calcium homeostasis. Additionally, the observed decline in CRP levels by 25% ($p<0.05$) signifies a reduction in systemic inflammation, which is often associated with CKD progression and cardiovascular risk.

These findings align with previous studies illustrating the efficacy of HDF in promoting renal function.[6,7]

The substantial reduction in serum levels of beta-2 microglobulin, creatinine, and blood urea nitrogen (BUN) following HDF treatment demonstrates the therapy's effectiveness in enhancing renal function and promoting the clearance of uremic toxins. The amelioration of these renal parameters is complemented by the observed enhancement in cardiac parameters, such as (LVEF) and reduction in (LVESD), indicating a potential cardioprotective effect of HDF in patients with advanced CKD.[8]

Furthermore, the study findings revealed a strong correlation between the improvement in anaemia parameters, such as haemoglobin levels, and the enhancement in cardiac function, emphasizing the crucial role of HDF in mitigating anaemia-related cardiac complications and improving overall cardiovascular health. The observed increase in haemoglobin levels following HDF treatment suggested an improvement in erythropoietin response and iron utilization, contributing to better oxygen-carrying capacity and enhanced myocardial oxygenation.[9]

The multifaceted benefits of HDF treatment, including the reduction in pro-inflammatory markers, such as C-reactive protein (CRP), and the improvement in calcium-phosphate metabolism, further support its potential to provide comprehensive cardiorenal protection in patients with advanced CKD. The attenuation of systemic inflammation and the maintenance of mineral-bone homeostasis through HDF may contribute to the reduction in cardiovascular risk and the prevention of adverse cardiovascular events in this patient population.[10]

Our study's findings underscore the importance of implementing integrated cardiorenal care strategies, such as HDF, to achieve optimal clinical outcomes and improve the overall quality of life in patients with advanced CKD. Further research focusing on the long-term effects of HDF on cardiorenal outcomes and its impact on reducing cardiovascular morbidity and mortality is warranted to validate our results and establish the therapy's role in comprehensive cardiorenal management.

In addition to the improvement in renal and cardiac parameters, our study identified several key factors contributing to the observed correlation between HDF treatment and cardiorenal protection. The substantial reduction in parathyroid hormone (PTH) levels following HDF therapy suggests a potential role in

preserving bone and cardiovascular health by regulating mineral metabolism and preventing secondary hyperparathyroidism in patients with advanced CKD.[11]

Moreover, the study results demonstrated a significant association between the enhancement in albumin levels and the improvement in various cardiac parameters, emphasizing the vital role of adequate nutritional status in promoting cardiac health and reducing the risk of cardiovascular complications in this patient population. The increase in serum albumin levels following HDF treatment reflects the therapy's ability to improve protein-energy wasting and enhance nutritional status, contributing to better cardiovascular outcomes and overall patient well-being.[12]

Furthermore, the observed reduction in potassium levels and the enhancement in potassium clearance following HDF treatment highlight the therapy's potential in managing electrolyte imbalances and preventing cardiac arrhythmias in patients with advanced CKD. The effective removal of potassium during HDF sessions contributes to the maintenance of optimal potassium levels and the prevention of life-threatening arrhythmic events, underscoring the therapy's crucial role in ensuring cardiac stability and reducing the risk of sudden cardiac death in this patient population.[13]

The cumulative evidence from our study strongly supports the comprehensive cardiorenal benefits of HDF treatment and highlights the therapy's potential to improve both cardiac and renal function in patients with advanced CKD. By addressing multiple pathophysiological processes associated with cardiorenal complications, HDF emerges as a promising therapeutic approach that can effectively mitigate the progression of cardiovascular and renal diseases, ultimately enhancing patient outcomes and prolonging survival in this vulnerable patient population. Further investigations focusing on the mechanistic underpinnings of HDF-mediated cardiorenal protection are essential to elucidate the therapy's precise therapeutic mechanisms and optimize its clinical applications in the management of patients with advanced CKD.[14]

4. Conclusion

B2M serves as a valuable marker of renal function and cardiovascular risk in patients with advanced CKD. The reduction in serum B2M levels following HDF treatment reflects the therapy's efficacy in improving renal function and mitigating cardiovascular risk, thus highlighting the potential of HDF as a comprehensive therapeutic approach for individuals with cardiorenal complications.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article

Funding

No Funds : Yes

Conflicts of interest

There are no conflicts of interest.

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