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Predictive value of osteoprotegerin and heart fatty acid binding protein as biomarkers for heart failure in chronic kidney disease patients on hemodialysis: A case-control study

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ABSTRACT: Heart failure (HF) is a major cause of mortality worldwide, especially among patients undergoing regular dialysis. The current research aimed to assess the potential of measuring osteoprotegerin (OPG) and HFABP levels as predictors for HF in a chronic kidney disease patients on hemodialysis in a case-control study that was conducted on 130 Iriqi patients with end stage renal disease (ESRD) undergoing hemodialysis who divided into two groups of hemodialysis patients: those without HF (group 1, n =80) and those with HF (group 2, n=50). The results revealed that age, Osteoprotogerin (OPG), Heart fatty acid binding protein (HFABP) , cholesterol, Trigelecirid (TG), Very Low Density Lipoprotein (VLDL) and Low Diensity Lipoprotein were higher in HF compared to the non-HF group. OPG and HFABP levels were more than doubled in the HF group, and these differences were highly statistically significant. Receiver operating characteristic analysis showed that OPG and HFABP performed well in differentiating between HF and non-HF patients, with area under the curve values of 0.922 and 0.860, respectively. Logistic regression revealed that OPG and HFABP were the strongest predictors of HF, with an odds ratio of 8.71 and 1.09, respectively. It was concluded that OPG and HFABP can be considered as biomarkers for predicting of HF in dialysis patients with OPG, in particularly, showed strong predictive value. These findings have implications for the development of improved diagnostic and management strategies for (HF) in ESRD.

KEYWORDS: Osteoprotegerin; HFABP; Heart Failure; Hemodialysis.

1. INTRODUCTION

It is common for Heart failure (HF) and kidney failure to coexist in the same individual [1]. It was reported that Chronic kidney disease (CKD) and HF have a reciprocal relationship, with HF significantly raising the likelihood of developing kidney failure [2] . It was also demonstrated previously that CKD and HF have reciprocal effects on each other. CKD can contribute to volume overload, while HF further exacerbates this condition. It is noteworthy that dialysis patients and coexisting Individuals with HF who initiate dialysis have a 2-year survival rate of 65%, which is lower than the 83% survival rate observed in those without HF [3]. In the population of HFrEF patients without advanced CKD, there is compelling evidence that demonstrates the efficacy of different medical treatments and specific devices. These therapies encompass B-blockers and medications that focus on targeting the renin-angiotensin-aldosterone system (RAAS) [4]. For this reason, we examine existing evidence for predicting HF in dialysis patients. The kidneys by studying everything related to vital indicators to find quick and immediate solutions to this serious disease

1.1. Osteoprotegerin as a Biomarker for Heart Failure

Osteoprotegerin (OPG) is a bone glycoprotein that belongs to the superfamily of tumor necrosis factor (TNFSF). OPG is primarily known for its role in the process of regulating bone remodeling in both normal physiological states and various clinical situations. Its main mechanism of action is to act as a "decoy receptor," meaning that it acts by preventing communication that occurs between the receptor activator of

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nuclear factor kappa-B (RANK) and receptor activator of nuclear factor kappa-B ligand (RANKL) [5]. RANKL is a critical activator of osteogenic differentiation and maturation, which is involved in the process of osteoclastogenesis. As a result, OPG prevents the maturation of bone cells and thus prevents bone formation. This leads to the prevention of activities associated with bone resorption [6]. OPG is found in different organs of the body (kidneys, bones, intestines, and stomach). In addition, they are generated by different types of cells such as (cells in the extracellular matrix, megakaryocytes, immune cells such as B lymphocytes) and cells in (vascular endothelium and vascular smooth muscle) [5]. In addition, several studies have shown that granulosa cells can release osteogenic hormone (OPG) in association with interlukin-17 (IL-17) [7, 8]. Activated T cells produce IL-4 and IL-13, which can stimulate OPG synthesis which plays an essential role within the process. of modifying bone metabolism and also contributes to the process of regulating stem cell activity, as well as the work of T and B cells [9]. New research suggests that OPG plays a role in cardiovascular disease (CVD) and can be considered as a predictive marker for it [10]. The mechanisms that regulate calcification in atherosclerosis are similar to those found in bone tissue. Initially. Recent studies have indicated a correlation between elevated OPG levels and a heightened susceptibility to CVD occurrences in individuals diagnosed with coronary artery disease (CAD) [11]. Further research has indicated that diabetic patients with increased OPG levels are more prone to developing atherosclerosis lesions and experiencing more severe calcification in their coronary arteries [12]. Additionally, patients with arterial hypertension also tend to have higher concentrations of OPG [6].

An elevated concentration of the OPG considered as a sign that indicating the existence of vascular disease associated with arterial hypertension. The specific mechanisms in which OPG might play a role in the progression of arterial wall damage and atherosclerotic plaque formation are not yet fully understood. However, OPG contributes to the promotion of inflammation within the blood vessel wall and promoting white blood cell adhesion to the endothelium [13]. Furthermore, the ability of angiotensin II, basic fibroblast growth factor (FGF), and platelet-derived growth factor to enhance the expression of OPG in vascular smooth muscle cells can aids in the formation of atherosclerotic plaques [10]. Research has confirmed that in people suffering from CKD and in a certain group of patients, there is a very clear relationship between the OPG/ tumor necrosis factor-related apoptosis-inducing ligand ratio and aortic pulse wave velocity (AoPWV), which is recognized as an indicator of vascular dysfunction occurring [14]. Several studies have demonstrated that individuals suffering from CAD exhibit markedly elevated levels of OPG in their blood plasma in comparison to healthy subjects. Within a cohort of patients afflicted by CAD, these increased OPG levels are correlated with a heightened prevalence of atherosclerosis and an elevated mortality risk, a finding that has been substantiated [15].

1.2. Heart Fatty acid binding Protein as a Biomarker for Heart Failure

Fatty acid binding proteins (FABPs) were initially identified as a cluster of cytoplasmic proteins that also classified into subtypes that found in different organ systems and at different concentrations [16] .. These proteins are low molecular weight (about fifteen kilo Daltons) [17]. The topic of FABPs has been widely discussed, particularly because of the association of HFABP with an increased risk of total and cardiovascular mortality. The Human Genome Organization (HUGO) Gene Nomenclature Committee recognizes 16 members of the FABP family, each encoded by a unique gene. Some well-known members include L-FABP (found in liver), I-FABP (found in intestine), HFABP (found in muscle/heart), A-FABP (found in adipocytes), E-FABP (found in skin), Il-FABP (found in ileum), B-FABP (found in brain), M-FABP (found in myelin), and T-FABP (found in testis). FABPs contribute to the cellular metabolism of fatty acids as they reversibly transporting long-chain polyunsaturated fatty acids (PUFA) from cell membranes to mitochondria, [16]. Among the major members of the FABP family, HFABP is perhaps the best known. It is also referred to as a breast-derived growth inhibitor which is present in tissues with a significant requirement for fatty acids such as the heart, skeletal muscle, brain, kidney, adrenal gland, mammary gland tissue, and blastocysts [18]. HFABP is abundant in the cytoplasm of striated muscle cells and is released during cardiac injury, as depicted in Figure 1. HFABP is present at elevated levels in both the ventricles (0.46 mg/g wet weight) and the atria (0.25 mg/g wet weight) compared to skeletal muscle. The expression of HFABP is regulated by a microRNA called miR-1, which may play a role in the pathogenesis of HF [19]. After the heart muscle undergoes damage, the release of HFABP occurs as the muscle cells release it into the bloodstream. This process is made possible by the small size of HFABP and its positioning within the cytoplasm. Additionally, it is believed that Transient increases in membrane permeability play a role in allowing HFABP to leak into the systemic circulation [20].

The purpose of the current research is to assess the potential of measuring OPG and HFABP levels as predictors for HF in a chronic kidney disease patients on hemodialysis.

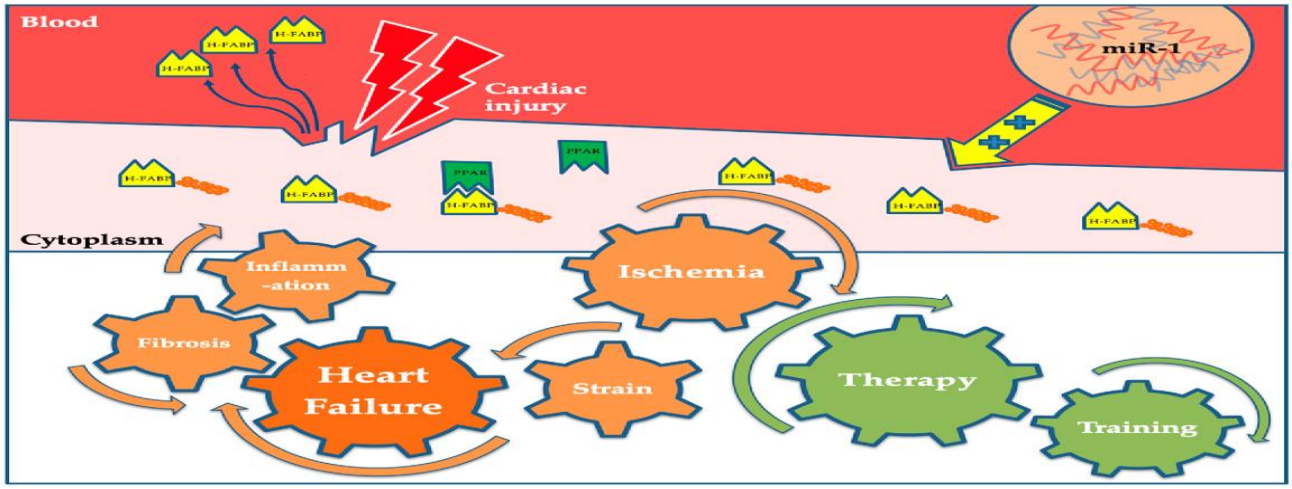

Figure 1. Role of HFABP under physiological conditions [16].

2. RESULTS

2.1. Analyzing clinical and biochemical factors in the Split HD-NHF and HD-HF

Statistical analysis was conducted to compare patients undergoing hemodialysis without heart failure (HD-NHF) with those undergoing hemodialysis with heart failure (HD-HF). The study examined various clinical and biochemical variables to identify any significant differences between the two groups.

Results illustrated in table 1 showed that the age of the HD-HF patients was significantly higher than those in HD-NHF patients. Results also revealed that the levels of OPG, HFABP, cholesterol, TG, VLDL and LDL were significantly higher in patients with HD-HF patients than those of HD-NHF patients whereas all other studied parameters in HD-HF patients were non significantly differ from those in patients with HD-NHF.

2.2. Receiver Operating Characteristic Curve Analysis:

The efficacy of the study biomarkers in differentiating between HD-NHF and HD-HF is summarized in Table 2, which includes the "AUC, optimal cutoff values, positive likelihood ratio (+LR), negative likelihood ratio (-LR), sensitivity, and specificity." The results revealed that the levels of OPG provides a high AUC value of 0.922 with an excellent sensitivity of 97.5% and very good specificity of 80% while HFABP lower values of AUC, sensitivity and specificity of 0.86, 73.75% and 86%; respectively in HD-HF patients against HD-NHF patients as illustrated in Figures 2 and 3

Table 1. Comparative analysis of all variables across HD-NHF and HD-HF groups.

Variables	Group	n	Range	Median	Mean± SD	SEM	P
Age	HD-NHF	80	67.00	54.50	52.40± 14.13	1.58	0.01
	HD-HF	50	50.00	59.50	58.34± 10.93	1.55	
BMI	HD-NHF	80	28.13	24.00	23.66± 4.32	0.48	0.31
	HD-HF	50	15.13	23.25	22.96± 2.83	0.40	
HD duration (months)	HD-NHF	80	149.0	50.0	52.80± 24.86	2.78	0.10
	HD-HF	50	115.0	36.0	44.92± 29.29	4.14	
OPG (ng/ml)	HD-NHF	80	2.52	1.64	1.56± 0.49	0.05	< 0.001
	HD-HF	50	6.03	3.14	3.22± 1.21	0.17	
HFABP (ng/ml)	HD-NHF	80	24.19	5.78	7.18± 3.99	0.45	< 0.001
	HD-HF	50	57.70	13.56	18.21± 11.22	1.59	
S.urea (mg/dl)	HD-NHF	80	152.13	150.49	145.26± 36.42	4.07	0.73
	HD-HF	50	129.38	142.47	147.29± 26.43	3.74	

S. Cr (mg/dl)	HD-NHF	80	13.93	9.13	9.69± 2.95	0.33	0.06
	HD-HF	50	9.46	8.35	8.52± 2.17	0.31	
S. Alb(g/dl)	HD-NHF	80	1.87	4.14	4.03± 0.41	0.05	0.28
	HD-HF	50	1.62	3.90	3.95± 0.42	0.06	
Cholesterol (mg/dl)	HD-NHF	80	142.0	145.00	142.70± 35.75	4.00	< 0.001
	HD-HF	50	139.0	187.00	179.66± 32.61	4.61	
TG (mg/dl)	HD-NHF	80	145.0	139.00	132.73± 39.77	4.45	0.01
	HD-HF	50	149.0	151.00	151.48± 32.26	4.56	
VLDL (mg/dl)	HD-NHF	80	29.00	27.80	26.55± 7.95	0.89	0.01
	HD-HF	50	29.80	30.20	30.30± 6.45	0.91	
HDL mg/dl	HD-NHF	80	25.50	39.00	37.69± 5.40	0.60	0.13
	HD-HF	50	37.30	38.95	39.46± 7.74	1.09	
LDL (mg/dl)	HD-NHF	80	109.90	82.10	78.47± 28.02	3.13	< 0.001
	HD-HF	50	126.30	109.75	109.90± 28.76	4.07	
RBCs)10^12/l)	HD-NHF	80	3.84	3.57	3.56± 0.59	0.07	0.79
	HD-HF	50	1.94	3.59	3.59± 0.43	0.06	
HCT (%)	HD-NHF	80	31.80	31.60	31.13± 5.52	0.62	0.90
	HD-HF	50	15.20	31.80	31.25± 3.94	0.56	
HGB (g/dl)	HD-NHF	80	6.20	9.45	9.32± 1.43	0.16	0.18
	HD-HF	50	4.20	9.75	9.64± 1.12	0.16	
WBCs(10^3/ul)	HD-NHF	80	7.00	5.35	5.62± 1.53	0.17	0.40
	HD-HF	50	12.80	5.20	5.36± 1.93	0.27	
PLT (10^3/ul)	HD-NHF	80	2007.00	216.00	245.65± 218.20	24.40	0.07
	HD-HF	50	348.00	187.00	186.94± 58.92	8.33	

Table 2. ROC Curve Analysis for the Diagnostic Performance of Biomarkers OPG and HFABP for Distinguishing Between Heart Disease States.

Variable		AUC	SE	95% CI	Cutoffs	Sensitivity (%)	Specificity (%)	+LR	-LR
HD-NHF vs HD-HF	OPG ng/ml	0.922	0.0262	0.862 to 0.962	≤2.262	97.50	80.00	4.88	0.031
	HFABP ng/ml	0.860	0.0329	0.789 to 0.915	≤7.823	73.75	86.00	5.27	0.31

SE: standard error, 95% CI: confidence intervals of the AUC

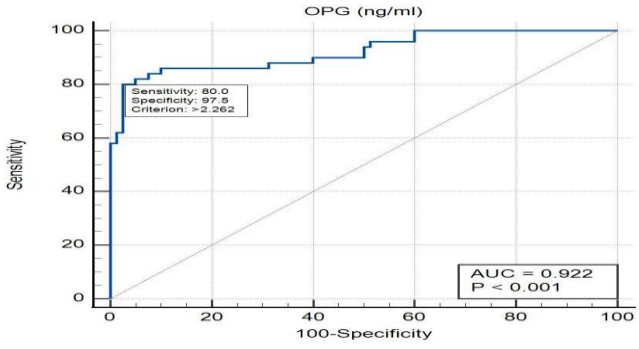

Figure 2. ROC curve analysis for OPG in distinguishing between HD-NHF and HD-HF patients.

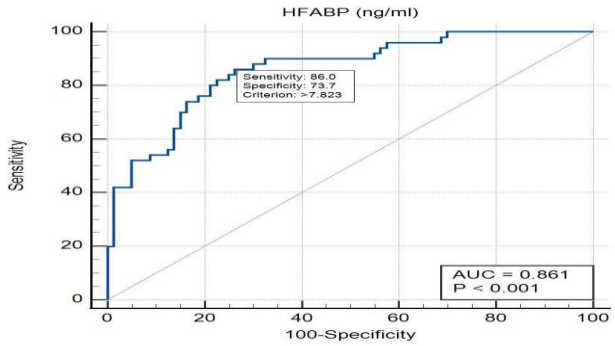

Figure 3. ROC curve analysis for HFABP in distinguishing between HD-NHF and HD-HF patients.

2.3. Logistic Regression Analysis:

Logistic Regression Analysis (LRA) was performed to evaluate the combined impact of OPG and HFABP on predicting HF in hemodialysis patients. The model was modified to account for gender, age and hemodialysis duration. The analysis, presented in Table 3, describes a binary logistic regression model that examined how the biomarkers affected the likelihood of classifying patients into the HD-HF category compared to the HD-NHF category. The overall model's statistical significance was determined using a Chi-square analysis using a 0.05 significance level (alpha). The results showed that the model was statistically significant ($\chi 2$ (3) = 98.78, p < 0.001), indicating that, collectively, OPG and HFABP Ssignificantly influenced the odds of a patient being classified into the HD-HF category. The goodness-of-fit of the model was assessed using McFadden's R-squared, which is a measure of model fit in logistic regression. The obtained McFadden R-squared value was 0.57, surpassing the threshold of 0.2 suggested by Louviere et al. (2000), indicating excellent model fit.

Table 3. Logistic Regression Results OPG and HFABP Predicting group HF in HD patient.

Variable	B	SE	$\chi 2$	p	OR	95.00% CI
(Intercept)	-8.00	1.43	31.52	< .001	-	-
OPG	2.16	0.66	10.78	0.001	8.71	[2.39, 31.68]
HFABP	0.09	0.07	1.63	0.202	1.09	[0.95, 1.24]

Note. **χ^2 (3) = 98.78, p < .001, McFadden R^2 = 0.57.3.**

3.DISCUSSION

3.1. The Impact of Age, HFABP, OPG, and Lipid Profile on Heart Failure in Dialysis Patients: A Comparative Analysis.

3.1.1. Age:

The current research was done to investigate the effect of age on HF among dialysis patients, particularly in the elderly population. The study investigation showed a significant difference in mean age between the HD-HF group and the HD-NHF group, as presented in Table 1. The observed age disparity is statistically significant, as indicated by a p-value of 0.01. This finding aligns with the results of a previous study conducted by Tang et al., 2023 who demonstrated that the advanced age is a well-known risk factors for HF in patients undergoing maintenance hemodialysis [21]. Furthermore, our findings are consistent with another study conducted by Yu and his co-workers in 2023 [22]. This elucidated that the risk of Hf in dialysis patients escalates with advancing age. Several factors contribute to this positive association between age and HF risk in dialysis patients. These factors include prevalent risk factors that become more common with age, such as CVD, diabetes, high blood pressure, and anemia. These comorbidities are frequently observed among dialysis patients [23]. Additionally, other factors such as left ventricular hypertrophy, chronic inflammation, and factors inherent to the dialysis treatment itself, like blood pressure fluctuations and electrolyte imbalances, may contribute to the increased risk of HF [24].

3.1.2. OPG

The mean OPG level in the HD-HF group is greater than the HD-NHF group, and the p-value is < 0.001, as shown in Table 1, which again highlights a very significant difference. Elevated OPG levels in the HD-HF group could be an indicator of underlying pathophysiological differences related to HF in dialysis patients. This is in agreement with many studies showing high serum OPG levels predict cardiovascular events in hemodialysis patients. OPG levels are associated with the severity of vascular calcification, age and

gender [25] and his result is in agreement with another study done by Collado et al., 2017 [26] which focused on the relationship between serum bone OPG levels and mortality, neurological morbidity, and cardiac function in patients undergoing hemodialysis.

Increased bone turnover: Osteoclasts, the cells that break down bone tissue, may be more active in HF patients, which could lead to an increase in bone turnover. Production of OPG may rise as a result obtained by Zotos and his colleagues in 2014 [27]. Inflammation from cytokines like TNF-alpha and IL-6 can stimulate OPG production [28]. High OPG may protect against bone loss from chronic inflammation. The presence of endothelial dysfunction in HF can be attributed to the production of OPG by endothelial cells. Elevated OPG levels can be a consequence of impaired endothelial function [29], RAAS activation is a prevalent occurrence in HF, while angiotensin II serves to enhance the production of OPG .The augmentation of angiotensin II levels may potentially result in an elevation of OPG [30]

3.1.3. HFABP

Through the results of the study obtained, shown in Table 1, it was shown that the mean level of HFABP in the HD-HF group is significantly higher than the mean level of HFABP in the HD-NHF group, and the p-value is < 0.001, which indicates a large variance. Elevated levels of HFABP in the HD-HF group may reflect cardiac stress or damage more commonly associated with HF. This is in agreement with many studies showing High serum levels of HFABP in the HD-HF group predict cardiovascular events [16], and another study showed the possibility of using HFABP as a diagnostic and prognostic biomarker in HF [31]

Other studies had shown elevated HFABP levels are correlated with pulmonary hypertension, right atrial hypertension, and worse clinical outcomes in HF patients [32]. Elevated levels of HFABP can be observed in individuals with HF is a complex condition where the heart's ability to pump blood is impaired, leading to various physiological changes. Several factors contribute to the elevation of HFABP in HF including -Damage to the heart muscle (myocardium). This can occur due to conditions such as cardiac injury. Pulmonary embolism, HF, cardiomyopathy, stroke, metabolic syndrome, and impaired glucose metabolism. When the heart muscle is injured, HFABP is released into the bloodstream as a result of cellular damage [33]. It was also noticed that the cellular stress is also involved in in release of HFABP as the individuals with HF experience significant stress due to the increased workload and impaired function of the heart which triggers the release of HFABP into the bloodstream, increases fatty acid metabolism in addition to chronic inflammation which is often present and can contribute to the release of HFABP [34].

3.1.4. Lipid Profile:

The mean concentrations of cholesterol, triglyceride (TG), Very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) experience fluctuations. In the HD-HF group are higher than the average levels in the HD-NHF group shown in Table 1. The statistical analysis, with p-values of < 0.001, 0.01, 0.01, and < 0.001, respectively, indicates that these differences between the two groups are statistically significant. This finding aligns with previous studies that have also observed elevated lipid profile levels in HF patients undergoing regular hemodialysis [35]. Patients frequently exhibit dyslipidemia, which is characterized by increased levels of TG, LDL-C, and VLDL, as well as a decrease in (HDL-C). [36]. Dyslipidemia is a condition where there are elevated levels of lipids in the blood, and it poses a significant risk to dialysis patients who also have HF [37]. This increases the chances of experiencing cardiovascular complications in the future. Several factors contribute to the disruption of lipid regulation in this specific population [38]. These factors include dialysis, insufficient dialysis sessions, inflammation, impaired kidney function, negative effects of medications, and genetic predisposition also plays a major role, in addition to insulin resistance, metabolic syndrome, and many other basic health problems [39].

3.2. Comparative Analysis of Osteoprotegerin and Heart Fatty Acid-Binding Protein

3.2.1. Receiver Operating Characteristic (ROC) Curve Analysis:

The results of ROC analysis indicate that both OPG and HFABP have good discriminative abilities in distinguishing between HD-NHF and HD-HF conditions. OPG has an AUC value of 0.922, indicating a high discriminatory power. The sensitivity of 97.50% suggests that OPG correctly identifies a large proportion of individuals with HD-HF. The specificity of 80.00% implies that OPG also performs reasonably well in correctly identifying individuals without HD-HF. The positive likelihood ratio of 4.88 indicates that a positive OPG result is nearly 5 times more likely in individuals with HD-HF compared to HD-NHF patients. Conversely, the negative likelihood ratio of 0.031 suggests that a negative OPG result significantly decreases the likelihood of HD-HF. The cutoff value of ≤2.262 allows for classification based on OPG levels. HFABP, on

the other hand, has an AUC value of 0.860, indicating a slightly lower discriminatory power compared to OPG. The sensitivity of 73.75% suggests that HFABP correctly identifies a lower proportion of individuals with HD-HF compared to OPG. However, the specificity of 86.00% indicates that HFABP performs well in correctly identifying individuals without HD-HF. The positive likelihood ratio of 5.27 suggests that a positive HFABP result is over 5 times more likely in individuals with HD-HF compared to HD-NHF. The negative likelihood ratio of 0.31 indicates that a negative HFABP result reduces the likelihood of HD-HF, although to a lesser extent than with OPG. The cutoff value of ≤7.823 is used for classification based on HFABP levels. As shown in Table 2 and Figure 3.

3.2.2. Comparison among all biomarkers in Logistic Regression Analysis

OPG, a protein responsible for regulating bone metabolism, has demonstrated potential as a predictive biomarker for HF in patients undergoing hemodialysis. It has been observed that OPG is involved in vascular calcification and inflammation, which are factors associated with HF [40]. Several studies have indicated that elevated OPG levels have been linked to heightened cardiovascular risk in different populations, such as individuals with CKD [41]. In the current research, the variable OPG has a coefficient of 2.16 which means that for every one-unit increase in OPG, the log odds of the outcome variable increase by 2.16. The p-value associated with OPG is 0.001, indicating that the relationship between OPG and the outcome variable is statistically significant. The odds ratio (OR) for OPG is 8.71, indicating that the odds of the outcome variable occurring is 8.71 times higher for each unit increase in OPG. The 95% confidence interval (CI) for the odds ratio is (2.39, 31.68), indicating the range within which we can be 95% confident that the true odds ratio lies. While the variable HFABP has a coefficient of 0.09. This means that for every one-unit increase in HFABP, the log odds of the outcome variable increase by 0.09.

4. CONCLUSION

It was concluded from the result presented that the levels of OPG and HFABP can be considered as good biomarkers for predicting HF in dialysis patients. OPG, in particular, showed strong predictive value. These findings have implications for the development of improved diagnostic and management strategies for (HF) in ESRD

5. SUBJECTS AND METHODS

5.1. Materiel and Instruments

The instruments that were used in this study and their companies were listed in Table 4.

5.2. Methods and Study design

A case-control study included 130 Iraqi patients suffering from end-stage renal failure and who were undergoing dialysis. Samples were taken from patients undergoing dialysis from the dialysis unit at Al-Imamian Al-Kadhimiya Medical City and Al-Karama Teaching Hospital in Baghdad, Iraq, between April 19, 2023, and December 12, 2023. The age range of the patients was between 18 and 75 years. Detailed information, including age, weight, and height, was collected from each patient. A 5 ml blood sample was taken from each patient. The blood sample was then divided into two tubes: 2 ml was placed in the first tube, which contained EDTA to estimate the complete blood count, and the remaining 3 ml of blood was placed in a gel tube. The gel tube was allowed to clot at room temperature for 30 minutes, and then the blood sample was divided into two tubes. Centrifuge it at 3000 rpm for 10 minutes to obtain the serum. OPG and HFABP levels were measured using separated serum and by enzyme-linked Immunosorbent assay (ELISA) technique. Other tests were also performed using serum, including urea, creatinine, albumin, and lipid profile (TG, Chol, HDL, LDL, and VLDL) and using appropriate colorimetric methods.

The patients were divided into two groups: Group 1, which consisted of 80 patients with ESRD undergoing regular dialysis without HF (43 males and 37 females), and Group 2, which consisted of 50 patients with ESRD. Undergoing regular dialysis with HF (30 males, 20 females).

Table 4. The instruments and their companies

Instruments	Supplied Company
Automatic hematology analyzer Sysmex	Germany
Centrifuge	U.S.A
Cobas C311 analyzer	Germany
Dimension EXL 200 Siemens for biochemistry	Germany
ELISA _Human Reader Microplate reader with 450 nm wavelength filter	Germany
fully auto analyzer for biochemistry smart	Germany
Incubator	Germany
fully auto analyzer for biochemistry smart	Germany

5.3. Statistical Analysis:

The study employed means and standard deviations or medians, and interquartile ranges to describe key variables across different groups: HD-NHF (Hemodialysis without Heart Failure), HD-HF (Hemodialysis with Heart Failure), and a control group. Comparative analysis was conducted using either one-way ANOVA or Kruskal-Wallis tests, depending on the data distribution, to compare means or medians among these groups. Subsequent post hoc analyses were performed to further explore specific group differences and identify statistically significant associations. Spearman correlation analysis was utilized to assess the relationships between different variables across the groups [42]. Receiver Operating Characteristic (ROC) Curve: are graphical representations that allow us to assess how well the biomarkers can distinguish between patients with and without HF. The AUC, or area under the ROC curve, is a numerical measure that indicates the generalized discrimination ability of each biomarker shown in Figures 2, 3, and 4. A higher AUC, closer to 1.0, suggests better diagnostic performance [43]. A significance level of $p < 0.05$ was considered statistically significant throughout the analyses. The statistical analyses were conducted using SPSS version 26 and Graph Pad Prism version 9 was utilized for graphical representations [44].

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