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ORIGINAL ARTICLE

The determination of heart failure with preserved systolic function by using Galectin-3 and other cardiac markers is affected by vitamin D status*

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ABSTRACT

Objective: Vitamin D affects heart muscle contraction through its direct and indirect effects on calcium and phosphorus metabolism. We aimed to investigate N-terminal pro-brain natriuretic peptide, high-sensitive troponin-I, galectin-3, fibroblast growth factor-23, suppression of tumorigenesis-2, and parathormone levels concerning Vitamin-D status in patients with heart failure with preserved systolic function.

Materials and Methods: Seventy-one patients diagnosed with heart failure with preserved ejection fraction and 61 healthy individuals as a control group were enrolled in the study. The relation between vitamin-D level and heart failure with preserved ejection and the determination of cut-off levels of all parameters was evaluated using ROC and logistic regression analyses.

Results: While vitamin-D levels in the patient group were lower than the controls (median 14.5 vs. 21.11 ng/ml, P=0.002), N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone levels in the patient group were significantly higher. The area under the curve values of these four elevated markers in the ROC analysis was significant for the diagnosis of heart failure with preserved ejection fraction. In the logistic regression analysis, a one-unit decrease in vitamin-D increased the risk of having heart failure with preserved systolic function 1,084 times, while a 10-unit categorical decrease in vitamin-D increased the risk 2,27 times. In the patient group, the area under the curves for N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone were higher when vitamin-D was >20 ng/ml compared to the group with vitamin-D < 20 ng/ml.

Conclusions: N-terminal pro-brain natriuretic peptide, galectin-3, suppression of tumorigenesis-2 and parathormone were affected by the vitamin-D status in determining heart failure with preserved systolic function, with increased detection capability when vitamin-D is >20 ng/ml.

Keywords: vitamin D, heart failure.

INTRODUCTION

Vitamin D (Vit D) influences cardiac muscle contraction through its various effects on calcium-phosphorus metabolism. Many studies have been conducted on this subject [1,2], and the relationship between systolic heart failure (HF) with vitamin D deficiency has been investigated. These studies did not find a significant link between vitamin D deficiency and heart failure and concluded that vitamin D supplementation does not provide a significant improvement in heart failure. Systematic reviews and meta-analyses have also shown that vitamin D supplementation has no positive effect on cardiovascular outcomes, including myocardial infarction and stroke. Meta-analyses also showed that vitamin D supplementation had no significant effect on cardiovascular risk factors (lipids, glucose, blood pressure) [3,4]. On the other hand, the effect of vitamin D on diastolic dysfunction of the heart has not been adequately studied.

Heart failure with preserved ejection fraction (HFpEF) describes patients who have a left ventricular ejection fraction (LVEF) greater than 50% and HF symptoms [5]. Various criteria are used to define HFpEF syndrome: (1) Clinical signs or symptoms of HF, (2) Evidence of preserved or normal LVEF, (3) Evidence of abnormal LV diastolic dysfunction as detectable by Doppler echocardiography or cardiac catheterization [6]. Diastolic dysfunction also seen in many people without HFpEF, is an important part of natural human aging. However, the presence of diastolic dysfunction is a risk factor for the development of HFpEF [7].

While echocardiography is used as the imaging method in the diagnosis and follow-up of HF, various plasma biomarkers are proven helpful in determining the diagnosis and prognosis of HF [8-10]. In recent years, many studies have investigated new HF biomarkers that may be useful in prognosis or grading. Although many biomarkers have been investigated, their practical application has been largely unsuccessful [11-15]. Although cardiac-specific biomarkers, including natriuretic peptides (Atrial and Brain Natriuretic Peptides) and High-Sensitive Troponin, are widely used in clinical practice, the benefits of other biomarkers have yet to be proven. Because they are influenced by general pathological processes such as cell death, inflammation and fibrosis these biomarkers are not specific to the heart or HF. To a large extent, these biomarkers cannot be linked to a single disease [15]. The levels of these markers are likely affected by vitamin D status in HFpEF because of the effects of vitamin D on myocardial function [1,2].

In this study, we investigated, for the first time, the relationship between the levels of HF markers and vitamin D status in patients with HFpEF. To this end, we measured N-terminal pro-brain natriuretic peptide (NT-ProBNP), high-sensitive troponin-I(HsTpn-I), galectin-3(Gal-3), fibroblast growth factor-23(FGF-23), suppression of tumorigenesis-2 (ST2) levels and vitamin D level in these patients in comparison with healthy controls. Since parathyroid hormone (PTH) and vitamin D levels are correlated with each other [16], hyperparathyroidism has various cardiac effects such as LV hypertrophy, hypertension, and diastolic dysfunction [17]; PTH was also included as a covariate in the analyses. The effect of vitamin D status on HF detection levels of these biomarkers in HFpEF was investigated by appropriate statistical methods.

MATERIALS AND METHODS

Patient selection and data collection

Our research was approved by Hacettepe University Ethics Committee in January 2021. The research was carried out in Hacettepe University Faculty of Medicine, Department of Internal Medicine (Endocrinology Division), and Department of Cardiology. Seventy-oneconsecutive patients (New York Heart Association (NYHA) class I, II [18]) attending inpatientand outpatient clinics with HFpEF and 61 healthy individuals without a diagnosis of HF formed the patient and control groups, respectively. The control group was selected from the hospital staff through face-to-face interviews. Vitamin D, HsTpn, NTproBNP, FGF-23, PTH, Gal-3, and ST2 serum levels were measured in both groups.

When selecting the participants, the patients with LV systolic dysfunction (EF < 50%) and those with various diseases that could cause cell death and inflammatory conditions such as infection, malignancy, and autoimmunity were excluded. Participants with kidney and liver failure, which

could potentially affect the marker level, were also excluded from the study.

The research was carried out between January 2021 and August 2021. The blood samples taken from the patients were centrifuged at 4000 rpm for 5 minutes, and serum samples were stored at -80°C until they were studied. After the samples were thawed in … University Biochemistry Department Laboratory, they were run with the Enzyme-Linked Immuno Sorbent Assay (ELISA) method(Diasource ® KAP1971-F1 Human 25 OH Vitamin D Total ELISA Kit, Cloud Clone-USCNK- ® SEA746Hu ELISA Kit for Human FGF23, Cloud Clone-USCNK- ® CEA866Hu Human PTH ELISA Kit, Cloud Clone-USCNK-® SEA485Hu Human N-Terminal Pro-Brain Natriuretic Peptide ELISA KIT, Cloud Clone-USCNK- ® HEA478Hu Human Cardiac Troponin I High Sensitive ELISA Kit, Cloud Clone-USCNK-® SEA303Hu Human Galectin-3 ELISA Kit, Cloud Clone-USCNK-® SEH820Hu Human ST2 (IL-33R, IL1RL1) Human ELISA Kit) (Human 25 OH Vitamin D, DIAsourceImmunoAssays SA, Belgium; Human, FGF23, Cloud-Clone Corp.,USA; Human PTH Cloud-Clone Corp.,USA; Human N-Terminal Pro-Brain Natriuretic Peptide, Cloud-Clone Corp.,USA; Human Cardiac Troponin I High Sensitive Cloud Clone Corp.,USA; Human Galectin-3 Cloud-Clone Corp.,USA; Human ST2 (IL-33R, IL1RL1) Cloud-Clone Corp.,USA).

Transthoracic echocardiographies of the participants, whose consent was obtained, were performed simultaneously (same day) with the collection of blood samples.

Statistical analysis

Frequencies and percentages for qualitative variables, mean ± standard deviation, or median (minimum-maximum) values for numerical variables were given as descriptive statistics. The means of normally distributed numerical variables in the groups with and without HFpEF were compared with the "student t test", and the distributions of the variables that did not show normal distribution were compared using the Mann-Whitney U test. Classification performances of biomarkers that may help to diagnose HFpEF were examined by receiver operating characteristic (ROC) analysis. At the end of this evaluation, the best cut-off points for diastolic heart failure markers were determined according to the Youden index criterion, and the sensitivity and specificity values, which are the basic test performance measures, were calculated for these points. The effect of biomarkers on heart failure was investigated with a multiple Logistic Regression Analysis along with vitamin D levels. Adjusting the effect of vitamin D level, the independent effect of biomarkers was evaluated with the odds ratio. The logistic regression model was created using the Backward Wald method.

Analyzes were reperformed for two subgroups, as vitamin D < 20 ng/ml and vitamin D > 20 ng/ml, according to vitamin D levels. Changes in the performance of biomarkers in each subgroup were examined by the area under the ROC curve. A p-value <0.05 was considered statistically significant in the study. Data were analyzed by using SPSS software (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp)

RESULTS

The distribution in terms of age, gender and body mass index in the patient and control groups is presented in Table 1.

The most common complaint in the patient group was dyspnea, followed by symptoms such as chest pain, edema, and palpitations. The patients in our patient group consisted of stage I and stage II HF patients according to the congestive heart failure classification of the NYHA. The control group was selected from asymptomatic healthy individuals.

Drug take in the control and patient groups is shown in table 2. As expected, the patient group takes more drugs in all drug groups.

The EF in the transthoracic echocardiograms of the controls was greater than 55% (mean 60.57%), with no evidence of diastolic dysfunction. The EF in the patient group was greater than 55 (mean 60.58%), and diastolic dysfunction was noted(When mitral flow indices were evaluated, the peak early [E]/late diastolic velocity [A] ratio was greater than 1 [19]).

Vitamin D, NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I, and ST2 levels were evaluated between the two groups using the Mann-Whitney U test due to the non-normal distribution of the data. Vitamin D, NTproBNP, Gal-3, PTH, and ST2 levels were statistically different between the two groups (Table 3).

Table 1. Distribution of control and patient groups in terms of gender, age, and body mass index (BMI)

	Controls (n=61)		Patients (n=71)	
Features	N	%	n	%
Male	29	47.6	36	50.7
Female	32	52.4	35	49.3
Age				
<40	22	36.2	4	5.6
40-49	24	39.3	18	25.4
50-60	15	24.5	26	36.6
>60			23	32.4
BMI (kg/m^2)				
Weak (<18,5)	1	1.6	0	0
Normal (18,5-24,9)	20	32.8	8	11.3
Overweight (25-29,9)	27	44.3	35	49.3
Obese (>30)	13	21.3	28	39.4

Table 2. Drug take in control and patient groups

	CONTROL (n=61)	%	PATİENT (n=71)	%
Angiotensin-converting enzyme inhibitörü	3	4,9	16	22,5
Angiotensin receptor blockers	3	4,9	19	26,8
Acetylsalicylic acid	5	8,2	21	29,6
STATIN	4	6,4	21	29,6
Beta Blocker	4	6,4	20	28,1
THIAZID	2	3,2	15	21,1
METFORMIN	2	3,2	15	21,1
Proton pompa inhibitörü	2	3,2	7	9,9
INSULIN	1	1,6	7	9,9
Sodium-glucose Cotransporter 2 Inhibitors	0	0	3	4,2
DPP-IV inhibitors	2	3,2	3	4,2
GLICLAZID	1	1,6	3	4,2
calcium channel blocker	1	1,6	11	15,5
Clopidogrel	2	3,2	5	7

NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I, and ST2 were evaluated by ROC analysis to determine whether they could be diagnostic in HFpEF and the cutoff values that provided the highest sensitivity for those that could (Figure 1).

FGF-23 and HsTpn-I levels between the two groups did not show a significant difference, and the area under the curve (AUC) values on the ROC curve were low. These two markers were not included in the subsequent calculations, and it was accepted that there was no significant difference between the HFpEF and control groups.

In ROC analysis, the best cut-off points were determined in terms of sensitivity and specificity for four markers (NTproBNP, Gal-3, ST2, PTH) according to the Youden index. As to this index, >2.92 value for Gal-3 (sensitivity 71% specificity: 68%) >27.87 value for NT-pro BNP (sensitivity 53%, specificity 75%), >9.22 value for ST2 (sensitivity % 53, specificity 73%) and a value >19.71 for PTH (sensitivity 50%, specificity 80%) were the most appropriate values for sensitivity and specificity. At these values, the highest sensitivity was in Gal-3, and the highest specificity was in PTH.

Four markers (the ones which are statistically different between the two groups) and vitamin D were evaluated by logistic regression analysis. Only Vitamin D, Gal-3, and NTproBNP were statistically significant.

The patient and control groups were divided into two groups according to their Vitamin D level as <20 ng/ml and >20 ng/ml. Twenty-seven patients in the control group and 50 patients in the patient group had a Vitamin D level of <20 ng/ml. Vitamin D level was >20 ng/ml in 34 patients in the control group and 21 in the patient group. In total, 41.7%

Table 3. Comparison of Vitamin D, NTproBNP, Gal-3, ST2, FGF-23, PTH, HsTpn-I, and ST2 results between control and patient groups

	Control(n=61) Median(Min-Max)	Patient(n=71) Median(Min-Max)	p-value
25-OH Vitamin D (ng/ml)	21.11 (5.97-57.39)	14.5 (6.73-34.62)	0.002
NTproBNP (pg/ml)	16.64 (0.35-319.76)	30.16 (1.8-1730)	0.007
Galectin-3 (ng/ml)	2.7 (1.15-7.13)	3.27 (1.53-27.86)	0.000
ST2(ng/ml)	6.22 (0.58-45.96)	9.91 (0.62-38.81)	0.015
PTH (pg/ml)	15.48 (4.09-78.69)	19.99 (4.13-49.68)	0.013
FGF-23 (pg/ml)	35.87 (25.27-306.19)	63.8 (33.83-265.03)	0.661
High Sensitive Troponin I (pg/ml)	8.15 (0.5-2800)	0.46(0.46-3055)	0.535

of the participants had a Vitamin D level >20 ng/ml, and 59.3% had a vitamin D level <20 ng/ml. Then, ROC analyses were performed for NTproBNP, Gal-3, ST2, and PTH in both groups to determine whether these markers were diagnostic in HFpEF. The AUC values were greater in the vitamin D-sufficient group for all four markers, but only Gal-3 AUC values were statistically significant in both groups (Table 4, Figure 2, 3).

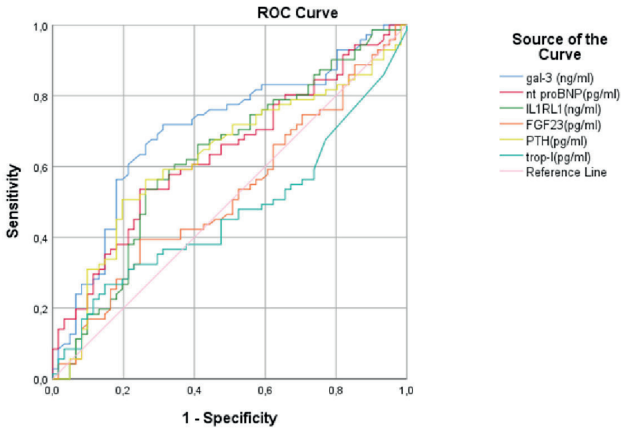

Figure 1. ROC curve results for NTproBNP, Gal-3, FGF-23, PTH, HsTpn-I and ST2.

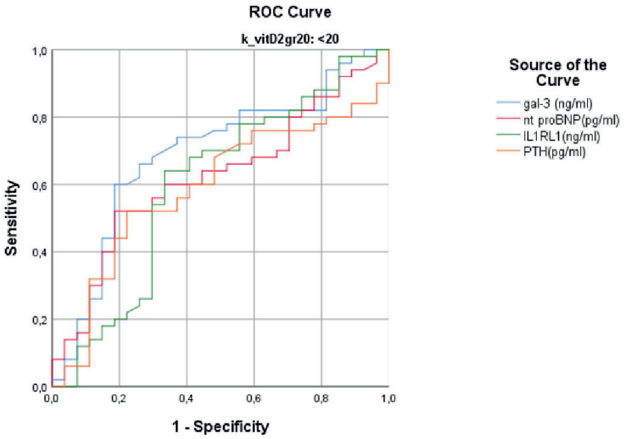

Figure 2. Evaluation of NTproBNP, Gal-3, ST2, and PTH levels by ROC curve in patients with vitamin D levels <20 ng/ml.

DISCUSSION

Our study is the first one in the literature investigating the effect of vitamin D on the detection levels of NTproBNP, Gal-3, ST2, FGF-23, PTH, and HsTpn-I for HFpEF. In our study, NT-ProBNP, Gal-3, ST2, and PTH were statistically significantly higher in the HFpEF group than in the control group. NTproBNP, Gal-3, ST2, FGF-23, PTH, and HsTpn-I were evaluated by ROC analysis to determine whether they could be diagnostic in HFpEF and the cut-off point values that gave the highest sensitivity for those that could be diagnostic. The areas under the curve(AUC) of Troponin-I and FGF-23 in the ROC curve were low and not statistically significant. Among NTproBNP, Galectin 3, ST2, FGF-23, and PTH, when evaluated without considering vitamin D levels, the highest AUC value was in Gal-3 and then NTproBNP. Vitamin D was significantly lower in the HFpEF group than in the control group. According to our logistic regression analysis results, having tenunits lower Vitamin D increases the risk of having HFpEF by 2.27 times. AUC values of NT ProBNP, Gal-3, ST2, and PTH

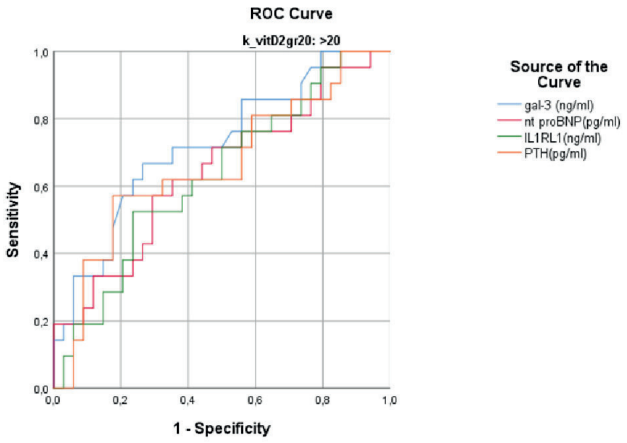

Figure 3. Evaluation of NTproBNP, Gal-3, ST2, and PTH levels by ROC curve in patients with Vitamin D levels >20 ng/ml.

Table 4. Evaluation of the areas under the curve according to the ROC curve when the patient and control groups were divided into two groups according to their vitamin D levels

Vitamin D level	Markers	AUC	p-value	95% Confidence Interval
<20 ng/m	gal-3 (ng/ml)	0.691	0.006	0.565-0.818
	ntproBNP (pg/ml)	0.623	0.077	0.495-0.750
	ST2 (ng/ml)	0.603	0.139	0.462-0.743
	PTH (pg/ml)	0.590	0.196	0.458-0.721
<20 ng/m	gal-3 (ng/ml)	0.721	0.006	0.582-0.861
	ntproBNP (pg/ml)	0.641	0.080	0.488-0.795
	ST2 (ng/ml)	0.632	0.103	0.481-0.782
	PTH (pg/ml)	0.658	0.050	0.505-0.812

in different vitamin D groups vary in ROC analysis for HFpEF diagnosis. For all four markers, the AUC values for the diagnosis of HFpEF were higher in the vitamin D>20 ng/ml group than in the vitamin D <20 ng/ml group. When Vitamin D was >20 ng/ml, NTproBNP, Gal-3, ST2, and PTH had an increased chance of success in diagnosing HFpEF. NTproBNP, Gal-3, ST2, and PTH were associated with Vitamin D status in determining ejection fraction preserved heart failure.

Markers that can be used in HF are affected by general pathological processes such as inflammation, fibrosis and cell death; therefore, they are not specific to the heart or HF. To a large extent, these biomarkers cannot be associated with a single disease [15]. In our study, we did not include those with various diseases that may cause cell death and inflammatory conditions such as infection, malignancy, autoimmunity, and those with kidney and liver failure, which could potentially be effective at the marker level.

In the PARAGON-HF Study published in 2018, the most common complaints in patients with HFpEF were dyspnea and fatigue [20]. The most common complaint was dyspnea in our patient group, as well.

Vitamin D deficiency may impair cardiac functions indirectly as well through PTH elevation. None of the participants included in our study (control group and HFpEF in both groups) had PTH elevations above normal. In addition, PTH was investigated in conjunction with other markers to elucidate whether it could be used as a marker in HFpEF. Interestingly, the specificity of PTH was higher than Gal-3 and NTproBNP when the optimal values for sensitivity and specificity were determined according to the Youden index for four markers that could be diagnostic in HFpEF (NTproBNP, Gal-3, ST2, PTH). Low PTH values may be more critical than NTproBNP and Gal-3 in excluding HFpEF. Lower PTH can be used to exclude the diagnosis of HFpEF in the future, but more clinical studies are needed on this subject.

In a study by Borbély et al., the cardiomyocytes of patients with HFpEF were harder than the control group [21] In our study, markers that could potentially reflect cardiac fibrosis, such as Gal-3 and ST2 [15], were higher in the HFpEF group. Vitamin D level is influential in determining heart failure with preserved ejection fraction may be explained by the fact that vitamin D deficiency is effective in cardiac diastolic dysfunction through fibrosis. On the other hand, Scragg et al. [22] showed that monthly high-dose vitamin D supplementation did not prevent cardiovascular diseases in 5000 patients. We believe that our study has indicated a possible relationship between vitamin D and fibrosis. However, more clinical studies are needed on this subject.

Plasma levels of natriuretic peptides are widely used in diagnosing patients with suspected HF and help evaluate patients with both systolic HF and HFpEF [23]. Normal natriuretic peptide levels largely exclude the presence of HF, particularly useful in acute situations to rule out HF [23,24]. Among the markers we investigated (NTproBNP, Gal-3, FGF-23, HsTpn, ST2), NTproBNP is the only marker used in clinical practice in HF. In our study, NTproBNP was significantly higher in the HFpEF group compared to the control group.

In a study by Tschöpe et al. [25] the median value of NTproBNP in the HFpEF group was 189.54 pg/ml, and the median value of NTproBNP in the control group was 51.89 pg/ml.The AUC was 0.83 in the ROC analysis performed for NTproBNP to diagnose HFpEF. At a cut-off value of 110 pg/mL, NT-proBNP showed 72% sensitivity and 90% specificity. Compared to our study, the median values were higher in both HFpEF and control groups. According to our study, AUC, sensitivity, and specificity values are higher. The differences in the results may be due to the wider differences between the minimum and maximum NTproBNP values in our study, which may be since we used ELISA as the measurement method. In the study of Polat et al. from Turkey on heart failure patients with preserved ejection fraction [26], NTproBNP was 617.75 pg/ml in the patient group while it was 66.35 pg/ml in the control group. In the ROC analysis, the AUC was 1, and the sensitivity and specificity for the 234 pg/ml value were 100%.Compared to our study, the differences may be since our study included more participants in both the patient and control groups, and our patients were in group I-II compared to the NYHA group, whereas the patients of Polat et al. [26] were NYHA II-III.

Many studies have shown that plasma levels of Gal-3 are associated with cardiac function [27]. In the review of Boer et al. [28], it was stated that Gal-3 is associated with various aspects of the pathophysiology of heart failure, particularly myocardial fibrosis, a transition from compensated to decompensated heart failure, and comorbidities such as kidney disease and diabetes. In a study by Kimmenade et al. [29], Gal-3 level was significantly higher in acute heart failure compared to the control group. Also, in that study, a high Gal-3 level effectively affected mortality. In our study, Gal-3 was higher in the HFpEF group than in the control group. The median Gal-3 value in our study was lower in both the patient and control groups compared to the value of Kimmenade et al. This result may be explained by the fact that the patient group in their study has presented with acute heart failure. In addition, both of our groups consisted of younger individuals compared to the study of Kimmenade et al. In the study by Polat et al. [26] the median serum Gal-3 level in patients was significantly higher than that of controls (5.35 vs. 0.51 ng/ml). A value of 1.79 ng/mL for galectin-3 had a sensitivity of 86.4%, a specificity of 100%, and an AUC of 0.98 in detecting HFpEF. Sensitivity, specificity, and AUC values are higher compared to our study. The differences between the two studies may be due to the strict exclusion criteria we have used. In addition, in the study of Polat et al., the patient and control groups were older than the ones in our study.

Our knowledge so far about the meaning of ST2 in HF is quite limited. Various studies have shown that the sST2(soluble ST2) isoform may play a role in cardiac fibrosis and remodeling [30]. On the other hand, the ST2L isoform has also been shown to be potentially cardioprotective by interaction with IL-33 [31]. We found higher ST2 levels in the HFpEF group than in the control group. In a study by Januzzi et al. [32] ST2 levels were measured in 593 patients with dyspnea who wereattending to the emergency department with and without acute destabilized heart failure. ST2 concentrations were higher in patients with acute heart failure compared to those without (0.50 vs. 0.15 ng/ml) [32]. In our study, ST2 levels were higher in both HFpEF and control groups. This difference may be since we worked with the group with preserved ejection fraction. In the study of Januzzi et al., no informationabout the patients' ejection fraction has been quoted.

The FGF-23 was found to be associated with left ventricular hypertrophy in a study by Faul et al. [33] Left ventricular hypertrophy has an important place in the etiopathogenesis of diastolic dysfunction. Roy et al. [34] found that mean FGF-23 was significantly higher in HFpEF patients than in the control group. In addition, high FGF-23 was associated with mortality and hospitalization at the end of the first year. In our study, FGF-23 was not significantly increased in the HFpEF group compared to the control group.

Limitations of The Study

We had only six patients with vitamin D above 30 ng/ml in a total of 132 participants evaluated (i.e., Vitamin D>30 ng/ml (proficiency) was not formed as a distinct group). The patients in our HFpEF group were predominantly NYHA class I and II. However, studies in the literature have been mainly conducted on decompensated heart failure patients. However, we think that the fact that we worked with a mildly symptomatic group also added a unique value to our study. Transthoracic echocardiography is a non-invasive, low-cost, and easily applicable method to evaluate the heart's systolic and diastolic functions. However, it may vary depending on the person's experience who performs it(We worked with an experienced cardiologist for echocardiography).In addition, the echocardiographic parameters to be interpreted for the diagnosis of HFpEF are complex, further complicating its evaluation.

In conclusion, NTproBNP, Gal-3, ST2, and PTH were affected by the vitamin D status in determining HFpEF, with increased detection capability when vitamin D is >20 ng/ml. Preserved ejection heart failure (HFpEF) is often difficult to diagnose, and using biomarkers to aid diagnosis is helpful in clinical practice. These biomarkers may reflect different aspects of HFpEF. Given the mixed pathophysiology of HFpEF, single biomarker may not be viable. We believe that it would be more accurate to use multi-marker approaches as in evaluating acute coronary syndrome.Prevention and treatment of vitamin D deficiency may be protective for HFpEF. However, extensive prospective clinical studies are needed to clarify this.

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Author contribution

The study was designed by AG and ME. Transthoracic echocardiographies of the patients were performed by SZ with the support and supervision of BE. Data collection was done by ME, IL, BGI, and SHO. Analysis and interpretation of the results were made by JK, AG and ME. The study was compiled into an article by AG and ME.

Ethical approval

The study was approved by Hacettepe University Non-Interventional Clinical Research Ethics Committee (Protocol no: 2021-02-02 /date 19.01.2021).

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Conflict of interest

The authors declare that there is no conflict of interest.

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