Galectin-3 as a Diagnostic Biomarker in Patients with Chronic stable Heart Failure

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Abstract
Heart failure (HF) is a progressive clinical syndrome resulting from various cardiac disorders. Galectin-3 promotes adverse cardiac remodeling leading to chronic heart failure (CHF). Currently, brain natriuretic peptide (BNP) and N-terminal pro BNP (NT-pro BNP) are widely used as diagnostic biomarker for heart failure (HF) and cardiac dysfunction in clinical medicine. This study aimed to measure the level of Galectin-3 as a diagnostic biomarker in patients with chronic heart failure (CHF) and controls to determine the association between galectin-3 levels with NT-pro BNP levels, age, gender, and left ventricular ejection fraction (LVEF). A case-control study was carried out from April 2022 to August 2022. A total of 60 patients with chronic heart failure (CHF) (patient group) and 60 subjects with normal ejection fractions (control group) confirmed by echocardiography were enrolled in Ibn Al-Bitar Specialized Center for cardiac surgery, Baghdad, Iraq. Enzyme-linked immunosorbent assay technique (ELISA) assessed serum NT-pro BNP and Galectin-3 levels. The median serum level of NT pro BNP in patients was 627.85 pg/ml (range= 366.89-4000 pg/ml) which was much higher than that controls (median = 262.63 pg/ml, range= 155.42-359.52 pg/ml) with highly significant differences. The mean serum level of Galectin-3 in patients with CHF was 8.96±1.75 ng/ml which was higher than that of controls (5.45±1.28 ng/ml) with a highly significant difference. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of NT-pro BNP and Galectin-3 in discrimination between patients with CHF and controls for NT-pro BNP, the area under the curve (AUC) was 1.0, 95%CI= 1.0-1.0, p <0.001. The sensitivity and specificity of the test at cut off value of NT-pro BNP= 363.21 pg/ml was 100% for both. For Galectin-3, the AUC was 0.944, 95%CI= 0.906-0.982, p <0.024. The sensitivity and specificity of the test at cut off value of Galectin-3 = 6.5 ng/ml was 93% and 85%, respectively. Galectin-3 with higher sensitivity and AUC can be used as a valuable biomarker for the diagnosis of CHF. Simultaneous testing of both Galectin-3 and NT-pro BNP can further improve the detection of patients with CHF.
Keywords: Galectin-3, NT-pro-BNP, Heart failure, biomarker, ejection fraction.

1. Introduction

Heart failure (HF), the leading cause of hospitalization in patients older than 65 years, is responsible for high mortality rates each year. Cardiac fibrosis is a pathological phenomenon in cardiac remodeling and is associated with heart diseases, including HF and cardiomyopathy [1].
Galectin-3, a member of the galactic family, is a 30 KDa protein. It is expressed intracellularly by inflammatory cells such as macrophages, neutrophils, mast cells, and fibroblasts [2,3]. Fibrosis and inflammation are principal mechanisms in heart failure development and cardiac remodeling [4]. Galectin-3 plays a principal role in fibroblast activation, is reportedly related to the development of cardiac hypertrophy and fibrosis [5,6].
Galectin-3 expression is increased in the remodeling myocardium, and it has been used as a prognostic biomarker in patients with heart failure [7]. Previous studies have shown that high circulating Gal-3 levels are indicative of the severity of heart diseases or related to increased risk of major adverse cardiovascular events including HF, arrhythmias or mortality [8–10].
B-type natriuretic peptides (BNP) are secreted by ventricular cardiomyocytes, and they reflect the severity of hemodynamic overload [11]. BNP levels are closely related to HF severity, and commonly used as a diagnostic and prognostic biomarker for HF [12]. BNP is linked to increased adverse cardiovascular
outcomes in heart diseases [13,14]. In addition, NT-pro BNP levels are strongly related to survival in HF regardless of ejection fraction [15].
Both BNP and NT-pro BNP are established as HF biomarkers and suggested for use by international guidelines [16,17]. We hypothesized that Galectin-3 might be related to NT-pro BNP levels in patients with chronic HF. Hence, we aimed to evaluate the galectin-3 and NT-pro BNP in chronic HF patients in this study

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2. Methodology

The study was executed during the term from April 2022 to August 2022 this study included 120 patients of adult male and female, and was divided into groups as follow:

Group I: 60 patients of adult male and female diagnosed by specialist cardiology having Heart Failure,it was also divided into:

A. about 30 sample patients having mild to moderate Heart Failure.

B. B: about 30 sample patients having severe HeartFailure .

Group II: 60 control sample apparently healthy subjects with no sign and symptoms of Heart Failure this was confirmed by a cardiologist, gender and age matched with patients' group will be included as controls in the study.

All samples were collected from Ibn Al-Bitar Specialized Center for cardiac surgery, Baghdad, Iraq.

This research was conducted in terms of the Declaration of Helsinki. The Institutional Review Board (IRB) at the College of Medicine of the Al-Nahrain University approved this study's protocol (Ref. IRB/148, Date: 19-4-2022). Accordingly, written informed consent was obtained from all the paricipants before the study.

Inclusion Criteria

- All adult with Chronic Stable Heart Failure patients.

Exclusion Crieteria

- Patient with (ACS) Acute Coronary Syndrom:
- (Classical ischemic Chest pain and or Troponin positive+).
- Acute H.F or Acute Decompensative Chronic H.F.
- Pregnant womens.

3. Methods And Materials

Preparation of blood samples

Collected blood samples from a patient with Chronic Stable Heart Failure and healthy people without this disease (for the biochemical Analyses) overnight fasting five milliliters obtained from each subject by venipuncture and put in the gel tube, by letting the whole blood tubes for 20-30 minutes at room temperature and transported the samples to laboratory unit and separated by centrifuge for 10 minutes at 6000 rpm. After separation of whole blood, the serum sample was drawn by pipette and put in clean and dry new microtube and stored in deep freezer (-20C°) until for the subsequent biochemical tests.

NT-Pro BNP, Gal-3 assessment Serum levels was be measured with the enzyme- linked immunosorbent assay (ELISA) technique.

4. Calculation of Body Mass Index (BMI)

Calculation of BMI was done by dividing the body weight in (Kilogram) by the square of the height (in meter), the equation for BMI: [18] BMI Mass (Kg)/ Height (m2).

5. Statistical Analysis

Statistical analyses were performed by using SPSS software version 25.0 (SPSS, Chicago). Continuous data were subjected to normality test (Shapiro Wilk test), Data with normally distribution were presented as mean and standard deviation and analyzed with Student t-test (for two groups comparison) or analysis of variance (ANOVA) (for three groups comparison). Data with non-normal distribution were presented as median and range and analyzed with Mann Whitney U test (for two groups comparison) or Kruskal Wallis (for three groups comparison). Categorical variables were expressed as number and percentage and analyzed with Chi-square test. Receiver operating characteristic curve (ROC) was used to evaluate the diagnostic value of NT-pro BNP and Galectin-3 in the context of discrimination between HF failure and healthy controls.

Pearson's correlation test was used to explore the possible correlation of NT- Pro BNP and Galectin-3 with other variables. A p- value less than 0.05 was considered to indicate a statistically significant difference.

6. Results

Demographic Characteristic of the study population

Table (1) shows the demographic characteristics of the study population. The mean age of patients with CHF was 57.67±12.54 years, which was very close to that of controls (56.08±12.32 years) with no significant difference. The two groups were identical in gender distribution (68.33% males and 31.67% females).

Although, patients demonstrated higher BMI than controls (27.03±2.93 kg/m2 vs.26.31±2.48 kg/m2), the difference was not significant. In contrast, 30% of patients with CHF were ex/current smokers compared with 5% of controls with a highly significant difference. Likewise, diabetes and hypertension were more common among patients' roles (55.67% and 28.33%, respectively) than controls (15% and 10%, respectively) with significant differences.

Table (1): Demographic characteristics of the study population

Variables	Patients (n=60)	Controls (n=60)	*p*-value
Age, years Mean ±SD Range	57.67±12.54 18-84	56.08±12.32 20-80	0.487
Gender Male Female	41(68.33%) 19(31.67)	41(68.33%) 19(31.67)	1.00
Body Mass Index, kg/m2 Mean ±SD Range	27.03±2.93 20.94-34.6	26.31±2.48 20.35-32.51	0.152
Smoking Never Ex/current	42(70%) 18(30%)	57(95%) 3(5%)	<0.001
Comorbidities Diabetes Hypertension	34(56.67%) 17(28.33%)	9(15%) 6(10%)	<0.001 0.011

Serum Levels of NT-pro BNP and Galectin-3

Data regarding serum level of NT pro-BNP were found to be non-normally distributed. Therefore, these data were expressed as median and, and non-parametric Mann Whitney U test was used to compare this marker groups between patients and controls. The median serum level of NT pro BNP in patients was 627.85 pg/ml (range= 366.89-4000 pg/ml) which was much higher than that controls (median= 262.63 pg/ml, range= 155.42-359.52 pg/ml) with highly significant differences (Figure 1). The mean serum level of galectin-3 in patients with CHF was 8.96±1.75 ng/ml which was higher than that of controls (5.45±1.28 ng/ml) with a highly significant difference (Figure 3-2).

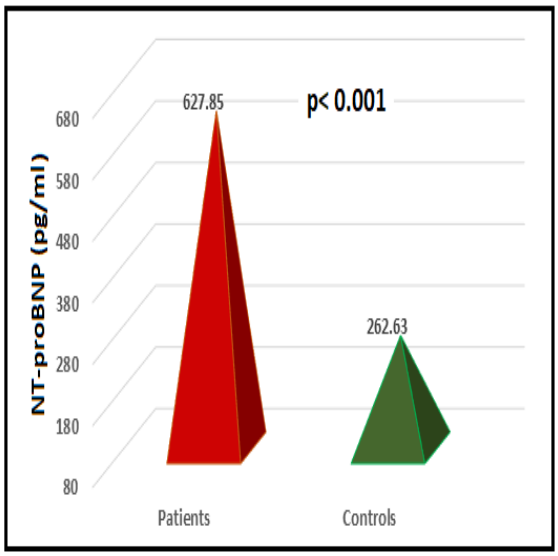

Figure 1: Median level of NT-pro BNP in patients and controls

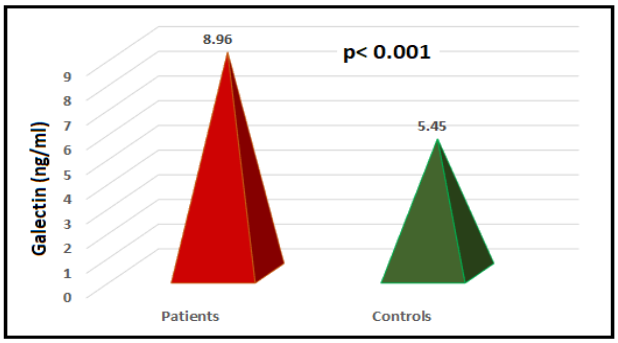

Figure 2: Mean level of galectin in patients and controls

Diagnostic Values of NT-pro BNP and Galectin-3

Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of NT-pro BNP and -3 in discrimination between patients with CHF and controls for NT-pro BNP, the area under the curve (AUC) was 1.0, 95% CI= 1.0- 1.0, p <0.001. The sensitivity and specificity of the test at cut off value of NT- Pro BNP= 363.21 pg/ml was 100% for both for galectin-3, the AUC was 0.944, 95% CI= 0.906-0.982, p <0.024. The sensitivity and specificity of the test at cut off value of galectin= 6.5 ng/ml was 93% and 85%, respectively (Figure 3, table 2).

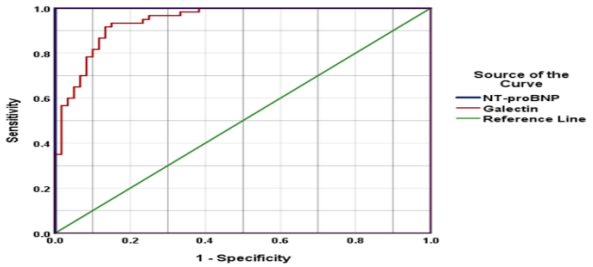

Figure 3: Receiver operating characteristic curve for NT-pro BNP and galectin-3 in discrimination between patients with CHF and controls

Table 2: Summary of diagnostic value for NT-pro BNP and Galectin-3 in discrimination between patients with CHF and controls

Marker	AUC, 95%CI	Sensitivity	Specificity	Cut off value	p-value
NT-Pro BNP	1.0, 1.0-1.0	100%	100%	363.21 pg/ml	<0.001
Galectin-3	0.944, 0.91-0.98	0.93	0.85	6.5 ng/ml	0.024

7. Discussion

HF is the final manifestation of all cardiovascular disorders. Multiple factors are involved in its evolution. Galectin-3 may be involved in the pathogenesis of HF. It has an advantage over the presently used markers as it does not vary with sudden hemodynamic changes [19]. Galectin-3 overexpression by macrophages was observed in heart failure and it is reportedly useful in the diagnosis and prediction of prognosis in HF patients [4,10]. The essential role of galectin-3 in HF was first described by Sharma et al. galectin-3 over-expression is detectable in macrophages during the early stages of myocardial dysfunction [4]. Galectin-3 induces fibroblast proliferation, leading to loss of systolic cardiac function [4,20] The present study estimated the levels of plasma galectin-3 and NT-pro BNP in CHF patients and controls. With respect to the participants' profile, cases and controls were matched for variables like age, gender, and BMI.

This study demonstrated two significant findings in patients with CHF. First, the galectin-3 and NT-pro-BNP levels were significantly higher in the HF group. Second, Galectin-3 and NT-pro BNP with higher sensitivity and specificity and AUC can be used as a valuable biomarker for the diagnosis of CHF. Simultaneous testing of both Galectin-3 and NT-pro BNP can further improve the detection of patients with CHF. In the present study, plasma galectin-3 and NT-pro BNP levels were markedly increased in HF than in the controls. Similar results were reported by Shi Y et al. [21], Jiang J et al. [22] and Barman et al [23]. Zhang et al showed that the mean value of NT-proBNP is significantly higher in HF patients versus healthy controls.

The ROC curve in the present study revealed that plasma galectin-3 was 93% sensitive and 85% specific, and plasma NT-pro BNP was 100% sensitive and 100% specific for diagnosing CHF. Galectin-3 levels in HF patients increased as their heart functioning worsened.

Daniels LB et al. [24] and Chen A et al. [25] have reported the prognostic importance of galectin-3 in the general population, independent of the established and frequently used marker, B-type natriuretic peptide (BNP)/NT pro-BNP. Amin HZ et al. have suggested that galectin-3 serves as an early mediator of fibrosis in the heart [19]. The FDA approved galectin-3 in 2010 for the assessment of HF together with clinical examination [26]. Galectin-3 has been identified as a novel marker reflecting fibrosis and would help in predicting adverse outcomes [26].

Shah RV et al. [27] and Djoussé L et al. [28] opined that galectin-3 measurement in HF patients provides additional diagnostic information and is also used to predict their prognosis.

8. Conclusions

This study demonstrates that galectin-3 and NT-pro BNP levels are significantly higher in patients with Chronic HF.

Galectin-3 has a distinct role in the pathogenesis of CHF. It is proven to be involved in the process of left ventricular remodeling. This study has also evidenced an increase in galectin-3 levels in the plasma of CHF patients when compared to the control group. Hence, higher concentrations of galectin-3 are predictive of CHF. Investigating galectin-3 levels during suspected CHF may have a role in early diagnosis, helping the physicians to achieve better therapeutic targets. Thus, it will improve the disease compliance of the patients and a future marker of research aiding disease-modifying therapy in CHF.

9. Disclousre

The authors declared no conflicts of interest. No funding was received for this study.

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