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Journal of Bioscience and Applied Research
www.jbaar.org

Diagnostic values of a model based on B-type natriuretic peptide, C reactive protein, and neutrophil-lymphocyte ratio for diagnosis of diabetic heart diseases patients

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DOI: 10.21608/jbaar.2021.201445

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Abstract

Background: People with type 2 diabetes means they are more likely to progress many complications such as hyperglycemia, low-grade inflammation, insulin resistance, and accelerated heart disease. Diabetic heart disease (DHD) can be diabetic cardiomyopathy, heart failure (HF), and coronary heart disease.

Methods: Blood samples from 100 patients with DHD, and 76 controls included [diabetic patients without cardiac diseases 56, and 20 healthy individuals were collected. C reactive protein (CRP) and neutrophil-lymphocyte ratio (NLR), vascular cell adhesion molecule (VCAM), B-type natriuretic peptide (BNP), were estimated in all study individuals. The area under receiver-operating characteristic curve (AUC) was used to evaluate the diagnostic accuracy of the single and combined markers. **Results**: Levels of CRP, BNP, and NLR had significant differences but VCAM had no significant differences among DHD, controls, diabetic, and healthy individuals groups. Then VCAM marker was excluded from further analysis. CRP was the most efficient biomarker among markers for discriminating DHD patients from healthy individuals, diabetic patients and, controls with AUCs were 0.99, 0.87, and 0.89; respectively. We developed a new model based on three blood markers (CRP, BNP, NLR) for differentiated DHD. Linear significant correlations were observed between model levels and candidate markers that included: CRP (r=0. 78; p <0.0001), NLR (r = 0. 0.53; p <0.0001), BNP (r = - 0.55; p <0.0001). The AUC of the model was 1.0 with 100 % sensitivity and 100 % specificity for discriminated patients with DHD from healthy individuals. For discriminate patients with DHD from diabetic patients, AUC was 0.90 with 92 % sensitivity and 81% specificity. For discriminate patients with DHD from controls, AUC was 0.93 with 92 % sensitivity and 83 % specificity.

Conclusion: The combination of three candidate biomarkers CRP, BNP, and NLR can be used to improve the diagnosis of DHD patients with high diagnostic performances.

KEYWORDS: Diabetes mellitus type 2, Biomarkers, Diabetic heart diseases.

Received: August 28, 2021. Accepted: October 19, 2021. Published: October 26, 2021

Introduction

Diabetes is a group of metabolic disorders distinguished by high concentrations of blood glucose. That's, results in the mutilation of the body's ability to utilize glucose due to either the pancreas does not produce insulin, or the body cannot use insulin suitably **(Refardt et al., 2020)**. The universal prevalence of diabetes mellitus type 2 has been gradually rising worryingly over many years **(Eizirik et al., 2020)**. Where 450 million adults with diabetes are excess across the globe, and this number was predictable to increase yearly **(Sultana et al., 2021)**. Several studies have shown that diabetes mellitus is responsible for a range of cardiovascular diseases, defined as diabetic heart disease such as heart failure, cardiomyopathy, coronary artery disease, etc **(Ritchie and Abe, 2020).** The cardiovascular diseases rate increased in patients with type 2 diabetes due to metabolic disorders and obesity **(Sultan et al., 2020)**. NLR is a new addition to the list of these inflammatory biomarkers. NLR is easy to obtain, low-cost, commonly available inflammation index, which can help in the prediction of patients with different cardiovascular diseases **(Afari and Bhat, 2016)**. Inflammation plays the main role in the pathogenesis of diabetes cardiac diseases. Inflammatory biomarkers (CRP and VCAM) can be used in the diagnosis of various cardiac diseases **(Pedersen, 2017; Liang et al., 2017)**. BNP is a member of a four-natriuretic peptide family, its blood levels are vital, not just as biomarkers of several cardiovascular deficiencies but also as an index of their severity **(Goetze et al., 2020).** The main aim of this study was the evaluation of the diagnostic value of biomarkers in diabetic heart diseases patients and the development of a model that can be used for discriminating DHD patients.

Patients and methods

Patients

In this study, the target population was individuals who have Type-2 DM with DHD (n=100), and diabetic patients without DHD (n=56) patients from the clinic of Diabetes of Alazhar University Hospital, New Damietta, Damietta, Egypt in addition to healthy individuals (n=20) i.e control group included 76 individuals. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Diagnosis of diabetes was done based on the American Diabetes Association criteria. Blood samples were obtained from each subject and collected in the morning after fasting for 12 hours and another sample was collected two hours after breakfast. Part of the samples was collected on K-EDTA as an anti-coagulant and analyzed directly for complete blood picture and glycosylated hemoglobin (HbA1c). The other part of samples were collected on plain tubes allowed to clot at room temperature then centrifuged to collect the serum. Samples of serum were stored at -20°c until analysis. The routine laboratory investigations were determined on an automated biochemistry analyzer (BT1500; Biotecnica instruments S.P.A, Italy). Complete blood pictures were performed using an automated hematology analyzer (Micros 60, Horiba Medical, Montpellier, France). NLR was calculated as a simple ratio between the absolute neutrophil and the absolute lymphocyte counts both obtained from the same automated blood sample at the admission of the study. HbA1c and CRP values were measured by using an automated analyzer (MISPA-i2, Agape, India). The levels of electrolytes were assayed by using Sensa-core, ST-200 plus, India. The levels of serum BNP and VCAM were assayed by using ELISA kit (Elabscience, Biotechnology Co. Lid, catalog NO; E-EL-H0598), (E-EL-H5587, Wuhan, Hubei, China), respectively.

Exclusion criteria: Subjects having type-1 diabetes mellitus were excluded, non-diabetic cardiovascular diseases and other causes of cardiovascular diseases.

Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) software, version

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22.0. The study variables were described as the mean ± standard deviation (SD) and P-value <0.05 was considered significant. Comparisons of parametric variables were done with ANOVA test, t-test while the Mann–Whitney U test was used in cases with nonparametric variables. The correlations between variables were analyzed with the Pearson correlation coefficient. Multivariate discriminant analysis (MDA) was used to develop a novel model. The diagnostic value of biomarkers was assessed by the area under the receiver operating characteristic (ROC). The best cutoff points were selected, and diagnostic powers were determined. Common indicators of model performance were derived from a 2 × 2 contingency table.

Results

Patients' characteristics

Demographic data and routine investigations of all patients and healthy individual groups are summarized in **Table 1**. With a mean age of 55±13.4 years and male 62 (35%) and female 114 (65%). Diabetes profile parameters (FBG, PPBG, and HbA1c) and lipid profile parameters (cholesterol, triglycerides, high-density lipoprotein- cholesterol (HDL), and low-density lipoprotein- cholesterol (LDL), liver profile, and kidney functions showed different significant differences between DHD, diabetic, controls, and healthy individuals groups as shown in **Table 1**. Using multivariate analysis showed that the increase in CRP, BNP, and NLR were significant (p< 0.0001) associated with the presence of DHD. On another hand, candidate biomarkers are summarized in **Table 2.** CRP, BNP, and NLR except VCAM have significant differences among DHD, controls, diabetic, and healthy individuals groups. VCAM marker was excluded from further analysis.

Diagnostic values of candidate biomarkers for discriminating DHD patients

The diagnostic value of three biomarkers (NLR, BNP, and CRP) was assessed by using the ROC curve. CRP was the most efficient marker (AUC = 0.99, 100 % sensitivity and 98 % specificity) for discriminant diabetics with DHD from healthy; for discriminant diabetics with DHD from patients with diabetic (AUC = 0.87, 84 % sensitivity and 86 % specificity) and for discriminant DHD from control individuals (AUC = 0.89, 84 % sensitivity and 88 % specificity).

Development of a novel model for discriminant between DHD

We developed a new model for differentiation between DHD based on three blood markers **(**CRP, BNP, NLR**)**. It can be calculated as ((0.558+ 0.027× CRP) + (0.026× NLR) – (0.003× BNP)). The levels of the model were increased with the severity of the disease. Its level was 0.01±0.16 in healthy individuals; 0.43±0.18 in diabetic individuals and 0.75±0.24 in DHD. Linear significant correlations were observed between model levels and candidate markers that included: CRP (r=0. 78; p <0.0001), NLR (r = 0. 0.53; p <0.0001), BNP (r = - 0.55; p <0.0001). The AUC of the model was 1.0 with 100 % sensitivity and 100 % specificity for discriminated patients with DHD from healthy individuals. For discriminated patients with DHD from diabetic patients, AUC was 0.90 with 92 % sensitivity and 81% specificity. Also, for discriminated patients with DHD from controls, AUC was 0.93 with 92 % sensitivity and 83 % specificity: **Table 3**.

Table 1. Comparison between levels of routine investigations of all patients and healthy individuals' groups:

Variables	**Healthy**	**Diabetic**	**Control**	**DHD**	**P-value***
FBG (mg/dl)	79.3±8.6	232.7±87.7	192.3±101	208.9±87.3	0.16
PPBG (mg/dl)	99.0±8.1	324.3±107.0	265±135	303.5±86.7	0.1
HBA1C (%)	5.1±0.3	8.5±1.6	7.7±2.0	8.4±1.4	0.03
Cholesterol (mg/dl)	174.8±14.5	170.5±28.8	171.6±25.7	166.2±36.4	0.57
Triglycerides (mg/dl)	86.0±14.6	175.2±57.4	151±63.5	174.3±69.2	0.1
HDL (mg/dl)	59.3±4.9	39.0±7.0	44.3±11.1	36.0±5.6	<0.0001
LDL (mg/dl)	98.3±14.5	96.7±27.2	97.1±24	95.3±30.8	0.82
ALT (U/L)	27.6±6	30.6±23	29.8 ±20	26.3±15	0.35
AST (U/L)	27.7±8.2	32.9±19	31.6 ±17	37.6±37	0.3
Albumin(gm/dl)	4.3±0.19	3.3±0.6	3.54±0.7	3.2±0.6	0.03
Bilirubin(mg/dl)	0.5±0.13	0.63±0.4	0.6±0.3	1.0±0.8	0.003
INR	1.05±0.06	1.1±0.18	1.1±0.16	1.3±0.9	0.08
Creatinine (mg/dl)	0.92±0.13	1.3±1.0	1.18±0.8	1.5±1.1	0.13
Urea (mg/dl)	30±4.0	56.3±57	49.4±50	64.8±51	0.16
Uric acid (mg/dl)	4.5±2.3	5.6±0.83	5.3±2.1	7.3±2.0	<0.0001
Sodium (mmol/L)	141.3±4.2	151.8±13.2	149.1±12.4	149.5±20.8	0.9
Potassium (mmol/L)	4.1±0.18	3.6±0.4	3.73±0.4	3.9±0.8	0.2
Calcium (mmol/L)	1.1±0.6	0.84±0.11	0.9±0.14	0.8±0.16	0.003

*Comparison between DHD vs control groups

Table 2. Comparison between levels of candidate biomarkers of all patients and healthy individuals' groups:

Variables	Healthy	Diabetic	Control	DHD	P-value*
Neutrophils (10^9/l)	**53.8±5.7**	72.6±6.6	67.7±10.5	77.2±9.7	<0.0001
Lymphocytes (10^9/l)	38.2±6.1	13.2±3.3	19.8±1.2	11.2±3.6	<0.0001
NLR (%)	1.5±0.3	6.2±2.6	4.9±3.1	8.1±4.2	<0.0001
CRP (mg/L)	2.2±1.0	6.6 ±4.4	4.1 ±1.3	12.9±11.7	<0.0001
BNP (pg/ml)	212.4±46.3	127.3±60.1	147.1±67	82±45.2	<0.0001
VCAM (ng/ml)	367.9±46.6	314.8±182.9	314.8±182.9	326.4±182.9	0.9

*Comparison between DHD vs control groups

Tables 3: diagnostic performance for single and combined markers:

Variables	AUC (95% CI)	P-value	Cut-off	Sensitivity	Specificity	PPV	NPV	Efficiency
DHD vs **Healthy**								
CRP (mg/L)	0.99	< 0.0001	4.0	100	91	98	100	98.3
NLR (%)	0.98	< 0.0001	4.1	98	91	98	90	96.7
BNP (pg/ml)	0.95	< 0.0001	164	96	88	100	97	97.1
CRP- NLR- BNP	1.0	< 0.0001	0.58	100	100	100	100	100
DHD vs **Diabetic**								
CRP (mg/L)	0.87	< 0.0001	6	84	86	92	75.4	85
NLR (%)	0.65	< 0.05	5.4	72	57	75	53.3	66.7
BNP (pg/ml)	0.73	< 0.001	106	84	62	80	69	76.3
CRP- NLR- BNP	0.90	< 0.0001	0.58	92	88	93	86	90.4
DHD vs **control (Diabetic + healthy)**								
CRP (mg/L)	0.89	< 0.0001	5.8	84	88	90	81	85.8
NLR (%)	0.74	< 0.001	5.1	82	58	72	71	71.6
BNP (pg/ml)	0.79	< 0.0001	105.5	84	70	76	76.8	77.8
CRP- NLR- BNP	0.93	< 0.0001	0.46	92	83	88	86.8	87.5

Discussion

Cardiac diseases are often clinically silent in diabetes and commonly are not discovered until the advanced stages of disease **(Marwick et al., 2018)**. The global prevalence of diabetes mellitus has given an increase to an epidemic of diabetes mellitus-induced HF. Although the significant research attention this phenomenon, termed diabetic cardiomyopathy **(Ritchie and Abel, 2020**). In this study, we represented the diagnostic values of some biomarkers for the investigation of diabetic heart diseases patients such as CRP, BNP, NLR, and VCAM. We reported that all biomarkers CRP, BNP, and NLR except VCAM can discriminate the diabetic patients with or without DHD from healthy individuals. Diabetes is a metabolic disorder, Much of this cardiac inflammation in the diabetic heart may be triggered or amplified by increased levels of inflammatory biomarkers, Infiltration of other inflammatory cells as neutrophils **(Ritchie and Abe, 2020)**. **Calle and Fernandez (2012)** have reported that CRP, a well-established, inflammatory biomarker strongly associated with diabetes. BNP is frequently used in the diagnosis of HF, chronic renal failure, type 2 diabetes, and acute coronary syndrome **(Maries and Manitiu, 2013)**. Furthermore, VCAM is considered an inflammatory marker for predicting cardiovascular diseases which have an important role in atherosclerosis **(Zhang and Mao, 2020)**. In our study, we assessed by using the ROC curve the diagnostics value of NLR, BNP, and CRP for discriminating DHD patients from diabetic patients, controls, and healthy individuals. CRP was the most efficient index among others with AUCs were 0.87, 0.89, and 0.99; respectively. Then NLR which has AUCs were 0.65, 0.74, and 0.98; respectively. Then BNP have AUCs were 0.73, 0.79, and 0.95; respectively. **Li et al (2018)** reported that CRP levels are elevated in DHD patients and correlate with the plaque composition like in patients who suffer from HF complications. In addition to increasing evidence suggests that CRP participates directly in atherogenesis. However, the level of BNP was assessed for a diagnosis of structural heart disease with AUC (0.77), sensitivity (61%), and specificity (92%) **(Nakamura et al., 2005)**. NLR has been proposed as a reliable biomarker of immune inflammation, activation, and oxidative stress injury, and, elevated for predicting mortality in cardiovascular disease patients, with AUC (0.76) was significantly higher than that of the neutrophil count (0.59) **(Turcato et al., 2019)**. Several studies were reported that AUCs of BNP, VCAM-1, and CRP were 0.89, 0.80, 0.76 for prediction of HF **(Lino DOC, et al., 2019; Stumpf et al., 2017; Steg et al., 2005). Al Aseri et al (2019)** reported that CRP at acute myocardial infarction had AUC (0.84), specificity (79%), and sensitivity (83%) to predict HF. In this study, to improve the discrimination of DHD from diabetic patients we developed a model based on the combination of CRP, NLR, and BNP, with high degrees of sensitivity, specificity, PPV, NPV, and efficiency were 92%, 88%, 93%, 86% and 90.4%; respectively. Several studies have been used a multimarker strategy and developed a score based on a combination of several biomarkers for the prediction of DHD **(Stengaard et al., 2017; Yanishi et al., 2016; O'Donoghue et al., 2016)**. **Konev et al (2020)**, showed that the AUC of the carboxy-terminal fragment of IGFBP-4 (CT-IGFBP-4), NT-proBNP, and CRP for the diagnosis of HF were 0.73, 0.68, and 0.67; respectively. Where the model based on a combination of these three biomarkers had AUC was 0.79.

In conclusion: CRP is the most sensitive marker for discriminant DHD patients. The three combined markers were the most efficient model for discriminant than of diabetic patients with DHD from diabetic patients without DHD.

References

Afari ME, Bhat T. (2016). Neutrophil to lymphocyte ratio (NLR) and cardiovascular diseases: an update. Expert Rev Cardiovasc Ther;14(5):573-7.

Received: August 28, 2021. Accepted: October 19, 2021. Published: October 26, 2021

Al Aseri ZA, Habib SS, Marzouk A. (2019). Predictive value of high sensitivity C-reactive protein on progression to heart failure occurring after the first myocardial infarction. Vasc Health Risk Manag;15:221-227.

Calle MC, Fernandez ML. (2012). Inflammation, and type 2 diabetes. Diabetes Metab;38(3):183-91.

Eizirik DL, Pasquali L, Cnop M. (2020). Pancreatic beta-cells in type 1 and type 2 diabetes mellitus: different pathways to failure. Nat Rev Endocrinol;16(7):349-362.

Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. (2020). Cardiac natriuretic peptides. Nat Rev Cardiol; 17(11):698-717.

Konev AA, Kharitonov AV, Rozov FN, Altshuler EP, Serebryanaya DV, Lassus J, Harjola VP, Katrukha AG, Postnikov AB. (2020). CT-IGFBP-4 as a novel prognostic biomarker in acute heart failure. ESC Heart Fail;7(2):434-444.

Li H, Sun K, Zhao R, Hu J, Hao Z, Wang F, Lu Y, Liu F, Zhang Y. (2018). Inflammatory biomarkers of coronary heart disease. Front Biosci; 10:185-196.

Liang KW, Sheu WH, Lee WJ, Lee WL, Fu CP, Wang JS. (2017). Differential expression of circulating vascular cell adhesion molecule-1 in subjects with coronary artery disease and cardiac syndrome X without known diabetes mellitus. Biomarkers; 22(8):798-804.

Lino DOC, Freitas IA, Meneses GC, Martins AMC, Daher EF, Rocha JHC, Silva Junior GB. (2019). Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. Braz J Med Biol Res; 52(12):e8658.

Maries L, Manitiu I. (2013). Diagnostic and prognostic values of B-type natriuretic peptides (BNP) and N-terminal fragment brain natriuretic peptides (NT-pro-BNP. Cardiovasc J Afr.;24(7):286-9.

Marwick TH, Ritchie RH, Shaw JE, Kaye D. (2018). Implications of Underlying Mechanisms for the Recognition and Management of Diabetic Cardiomyopathy. J Am Coll Cardiol; 71:339–351.

Nakamura M, Tanaka F, Sato K, Segawa T, Nagano M. (2005). B-type natriuretic peptide testing for structural heart disease screening: a general population-based study. J Card Fail;11(9):705-12.

O'Donoghue M L, Morrow D A, Cannon C P, Jarolim P, Desai N R, Sherwood M W, Murphy S A, Gerszten R E, Sabatine M S. (2016). Multimarker Risk Stratification in Patients with Acute Myocardial Infarction. J Am Heart Assoc; 5, e002586.

Pedersen BK. (2017). Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest; 47(8):600-611.

Refardt J, Winzeler B, Christ-Crain M. (2020). Diabetes Insipidus: An Update. Endocrinol Metab Clin North Am; 49(3):517-531.

Ritchie RH, Abel ED (2020). Basic Mechanisms of Diabetic Heart Disease. Circ Res; 22; 126(11):1501-1525.

Steg PG, Joubin L, McCord J, Abraham WT, Hollander JE, Omland T, Mentré F, McCullough PA, Maisel AS. (2005). B-type natriuretic peptide and echocardiographic determination of ejection fraction in the diagnosis of congestive heart failure in patients with acute dyspnea. Chest;128(1):21-9.

Stengaard C, Sørensen J T, Ladefoged S A, Lassen J F, Rasmussen M B, Pedersen C K, Ayer A, Bøtker H E, Terkelsen C J, Thygesen K. (2017). The Potential of Optimizing Prehospital Triage of Patients with Suspected Acute Myocardial Infarction Using High-

Sensitivity Cardiac Troponin T and Copeptin. Biomarkers; 22(3–4): 351–360.

Stumpf C, Sheriff A, Zimmermann S, Schaefauer L, Schlundt C, Raaz D, Garlichs CD, Achenbach S. (2017). C-reactive protein levels predict systolic heart failure and outcome in patients with first ST-elevation myocardial infarction treated with coronary angioplasty. Arch Med Sci;13(5):1086-1093.

Sultan A, Singh J, Howarth FC. **(2020).** Mechanisms underlying electromechanical dysfunction in the Zucker diabetic fatty rat heart: a model of obesity and type 2 diabetes. Heart Fail Rev; 25(5):873-886.

Sultana R, Mughal UR, Ghoto MA, Muhammad SM, Abbas J, Laghari SH, Mughal Z, Qureshi Y, Jameli J, Khan T. (2021). Awareness and Lifestyle Practices among Type-II Diabetics Pertaining to the Disease Attended at Outpatient Clinics of Nawabshah, Shaheed Benazirabad. J Pharm Res Intern; 67-75.

Turcato G, Sanchis-Gomar F, Cervellin G, Zorzi E, Sivero V, Salvagno GL, Tenci A, Lippi G. (2019). Evaluation of Neutrophil-lymphocyte, and Platelet-lymphocyte Ratios as Predictors of 30-day Mortality in Patients Hospitalized for an Episode of Acute Decompensated Heart Failure. J Med Biochem;38(4):452-460.

Yanishi K, Nakamura T, Nakanishi N, Yokota I, Zen K, Yamano T, Shiraishi H, Shirayama T, Shiraishi J, Sawada T, Kohno Y, Kitamura M, Furukawa K, Matoba S. (2016). A Simple Risk Stratification Model for ST-Elevation Myocardial Infarction (STEMI) from the combination of blood examination variables: Acute Myocardial Infarction-Kyoto Multi-Center Risk Study Group. PLoS ONE; 11 (11), e0166391.

Zhang L, Mao H. (2020). The relationship between serum VCAM-1 and Alzheimer's Disease in patients with type 2 diabetes mellitus. Diabetes Metab Syndr Obes., 30;13:4661-4667.