Original article

Risk factors and Relationship of Cardiac Biomarkers with Cardiovascular Disease in Type-2 Diabetic Among Palestinian Patients-Gaza Strip

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Abstract

The aim of the present study is to investigate the relationship of risk factors and cardiac biomarkers level with the development of cardiovascular disease (CVDs) in type-2 diabetic Palestinian patients in Gaza strip.

Methodology; Study population consists of three groups: control (group 1); (group 2) with an equal number of diabetic type-2 without Cardio Vascular disease "CVD". Both groups were attending Al- Remal central clinic. (group 3) are diabetic type-2 at the Cardio Care Unit (CCU) at El- Shifa hospital, Gaza. The study was conducted at the laboratory of Central laboratory in Remal Clinic, Gaza Strip-Palestine from June to October 2018 and included 180 subjects divided to the three equal groups: control, Type-2 Diabetic Patients "T2DM" an T2DM with CVD, age of the three groups was 45 – 65yrs. A questionnaire and vacationer serum blood samples were collected from all participants for the laboratory analysis.

Results: The results revealed a significant difference between the three studied groups in the gender ($P < 0.05$), BMI, smoking, and level of hypertension "systolic/diastolic" significant difference ($P< 0.01$). As for the age, there was no significant difference among studied groups. A statistical significant difference ($P<0.01$) was clear between diabetes duration and CVD complication in "T2DM" patients, also for age at onset of DM for T2DM without CVDs groups and for T2DM with CVDs groups; ($P<0.05$). Significant difference was evident in the level of serum Lactate Dehydrogenase LDH, Creatine Kinase "CK", Creatine Kinase Muscle Brain, Creatine Kinase Muscle Brain "CKMB" and Terminal Brain Natriuretic Peptide **"**NT-BNP" among three studied groups ($P<0.01$).

Conclusion: There are many risk factors that have effect on cardiovascular disease in T2DM; using cardiac biomarkers that may aid in the early diagnostic and prognostic evaluation of high risk patients with T2DM to avoid complication to CVDs.

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Introduction

Diabetes mellitus is a serious global cause of mortality and morbidity with significant raises in the prevalence and number of cases in the last 30 years (Seuring *et al*., 2016). The first WHO Global report on diabetes demonstrates that the number of adults living with diabetes has increased four folds since 1980 to reach 422 million adults. This dramatic rise is largely attributed to the rise in type- 2 diabetes (WHO Global report on diabetes, 2016). The prevalence of diabetes

in the Palestinian population in the West Bank, Gaza and East Jerusalem is (15.3%) compared to a worldwide prevalence of 6%. 4.4% of the total diabetic population in Palestine, were diagnosed with type 1 diabetes, 95.3% are diagnosed with type 2 diabetes. (Palestine National Diabetes Program, 2016).The incidence of diabetes in 2017 in Gaza strip was 264.8/100,000 people, which increased by 7.21% for the year 2016. (Annual report chronic disease/ Gaza strip, 2017)

In 2012, there were a total of 3.7 million deaths, of which 34% were in age groups below 70 years, 1.5 million deaths were caused by DM and 2.2 million by poorly controlled blood glucose, which increased the risks of cardiovascular. The percentage of deaths under the age of 70 years caused by elevated blood glucose is higher in low and middle-income countries than in high-income countries (WHO Global Report on Diabetes, 2016). Risk factors and elevated cardiac biomarkers LDH, CK, CK-MB, and NT-BNP have been demonstrated to be important prognostic determinants of patients with diabetes to identify high risk patients **(**Bodor, 2016), especially NT-BNP as novel biomarkers (Wang *et al*., 2017) which mediate various cardiovascular effects and On the one hand, secretion of NPs is resulting in stretch of the cardiac wall and volume overload of cardiac cavities (Goetze *et al*., 2016). NT-BNP had a biphasic association with T2DM in which the risk of diabetic incident decreased by evaluation physiological range of NT-BNP level (Sanchez *et al*., 2015). Patel, 2014 aimed to assess cardiac biomarkers, LDH.CK and CK-MB, and NT-BNP in diabetic and non-diabetic patients with myocardial infarction, with demographic factors; sex, age, hypertension and smoking. Ali *et al*. (2016) evaluated the activity of cardiac enzymes in diabetic and non-diabetic acute myocardial infarction patients. Berezin, (2017) reviewed our knowledge regarding clinical implementation of the biomarker-based strategy of the cardiac risk assessment in T2DM patient population. Gidding, et *al.,* (2018) examined cardiac biomarkers over time in youth-onset type 2 diabetes, and relate serum concentrations to cardiovascular disease risk factors.

Material and methods

Case-control study design This study was conducted in the laboratory of Central laboratory in Remal Clinic, Gaza Strip-Palestine in the period from March to October 2018. The study included 180 subjects divided to the three groups: 60 as control, 60 T2DM patients and 60 T2DM with CVD, matching for age between three studied groups aged 45 to 65 years. The serum and EDTA blood samples were collected from all participants for the laboratory analysis. The study was conducted in Gaza strip- Palestine at the Central laboratory of Al-Remal clinic. The present study was conducted during the period from June to October 2018.

The study population consisted of type 2 diabetic patients without CVD complication recruited from the outpatient clinic at the Diabetic Department of Remal Clinic Center in Gaza Strip and type 2 diabetic patients with CVD complication recruited from the Cardio Care Unit (CCU) at El- Shifa Hospital, Gaza Control group was equal number of an age matched. All of diabetic type-2 patients with or without CVD were included in the study. While patients with liver diseases, Type 1 diabetic patients and Patients with kidney failure are excluded in this study.

180 subjects, their age ranged from (45-65) years, were selected and divided into three groups: group 1 include 60 healthy subjects “control”, group 2 comprise 60 patients type 2 diabetic without cardiovascular disease recruited from the out patients clinic at the Diabetic Clinic Center and group 3 include 60 subjects 2 diabetic with cardiovascular disease recruited from the Cardio Care Unite at El-Shifa (CCU) at El-Shifa Hospital, Gaza .A meeting interview was used for filling in a questionnaire which designated for matching the study need. All interviews were conducted face to face by the researcher herself. The questionnaire included questions on demographic and clinical data .Venous blood samples were collected (about 5 ml) was drawn into serum vacationer tubes for LDH, CK and CKMB by chemistry auto-analyzer (Erba, 200) and NT-BNP by

Eliza kit (R & D company). These biochemical tests done in the laboratory in Remal clinic.

Ethical approval:

Permission for the study was obtained from Helsinki committee in the Gaza Strip in 2/10/2017 and administration approval was obtained from the human resource development general directorate in the Ministry of Health. Moreover, consent informed were gained from all participants before enrolment in the study after explained study's objectives and anonymity, right and confidentially were saved.

Statistical analysis:

Statistical analysis was conducted using Statistical Package for Social Science (SPSS) version (22) soft-ware USA for windows. Descriptive statistics will be reported as Mean ± SD and ANOVA test P values (P <0.05) will be considered statistically significant. Sample distribution of the study variables and the cross tabulation were applied. Descriptive was described by mean ± standard deviation (SD) and range as minimum and maximum values was used.

Results and Discussion

1. Descriptive variables in the three studied groups

Table (1) summarizes the general demographic variables of the three studied groups were contains 58.3 % males and 41.4 % females in control, group 1, in T2DM without CVDs group 2 were contain 63.3% males and 36.7%, while T2DM with CVDs group 3 contains (38.3%) male and 61.7%. The means age for the three were 53.8 ± 6.5, 53.9 ± 5.6 and 55.6 ± 6.9. There was no significant difference according to age; where the study sample matched for age. With regard to BMI the mean were 26.9 ± 3.2, 32.2 ± 6.6 and 32.4 ± 6.9 kg/m^2 for control, T2DM without CVDs and T2DM with CVDs respectively. ANOVA test clarified that there was significant difference (P < 0.001) in the studied group according to BMI. Post hoc elucidated that there was a significant difference between group 2 and group 3 (P<0.001) compared with group 1, whereas no significant difference between group 2 and group 3. add to in smoking variable most participants are non-smoker category but it found 3.3 %, 11.7 % and 28.3% of participants in the three studied groups respectively are current smoker and ANOVA test defined that there was significant difference among studied groups (P<0.001) and post hoc has been appointed that there was a significant difference between group 2 and group 3 (P <0.001) in comparison with group 3 and no significant difference between group 2 and group 3. On the light of the present results, no significant difference was found among gender and age, add to smoking variable most participants are non-smoker category but it found small proportion of participants in the three studied groups respectively are current smoker and ANOVA test defined that there was significant difference among studied groups (P<0.001) and post hoc has been appointed that there was a significant difference between group 2 and group 3 (P <0.001) and no significant difference between group 2 and group 3., these results of BMI and smoking among studied groups Conversely other studies found study show significant difference between BMI and smoking whereas similar to present study results unless. The reason for these conflicting results may be due to the variation in the sample size from study to other. As for the mean levels of systolic/diastolic blood pressure were 114.8 ±5.7/128.8 ±15.7, 126.3 ±8.7 and 74.3 ±6.1/84.3 ±11.4/ 81.7 ±6.2 for group 1, group 2 and group 3 respectively. ANOVA test clarified that there was significant difference between three studied groups (P<0.001); post hoc explained that there was significant difference in group 2 and group 3 in compared with group1, while there was no significant difference between group 2 and group 3 in systolic / diastolic blood pressure.

2. Duration of diabetes in the Patients groups

Table (2) and Figure (4) show the mean values of diabetes duration and age at onset of DM between T2DM without CVDs and T2DM with CVDs. The mean values of diabetic duration and age onset of DM were 6.7 ± 4.9 years and 11 ± 9.2 years ; 47.9 ± 7.1 years and 49.7 ± 9.5 years in the group 2 and 3 , respectively. While the mean age at onset of DM was 47.9 ± 7.1and 49.7± 9.5 for group 2 and 3, but regarding the mean age of onset CVDs was 53.5 ± 5.9. Results of the study clarified that there was a significant relationship between diabetes duration and CVD complication in T2DM patients. There was an

increase trend in the duration of diabetes, such increase was statistically significant (P<0.001).

Table (1): Demographic variables in the three studied groups

Variables	Group 1 Control (%)	Group 2 T2DM (%)	Group 3 T2DM & CVD (%)	F	*P*
Gender Males Females	 58.3% 41.7%	 63.3%[b] 36.7%	 38.3% 61.7%	4.352	0.014*
Age (years) Range	53.8 ± 6.5 (45-65)	53.9 ± 5.6 (45-65)	55.6 ± 6.9 (45-65)	1.638	0.197
BMI (kg/m^2) Range	26.9±3.2[ab] 22-33.4	32.2±6.6 (19-44.9)	32.4 ± 6.9 (23-57.8)	17.559	0.000**
Smoking Non-smoker Past-smoker Current-smoker	 91.7 5.0 3.3	 56.7[a] 31.7 11.7	 56.7[b] 15 28.3	11.955	0.000**
Systolic blood pressure (mmHg) Range	114.8 ±5.7 (100-120)	128.8±15.7 (105-180)	126.3 ±8.7 (100-150)	27.999	0.000**
Diastolic blood pressure (mmHg) Range	74.3 ±6.1[ab] 60-80	84.3 ±11.4 65-130	81.7 ±6.2 70-95	23.232	0.000**

[a ,b, c]: significant at P≤0.05)

This indicates that duration of DM was risk factor for CVDs complication development in T2DM patients. Martín et al., (2014) showed that duration of DM had increases the risk of CHD death independent of coexisting risk factors. furthermore, Gimeno *et al.*, (2014) demonstrated diabetes duration superior to 15 years significantly increased cardiovascular risk of the patients and they concluded that it could be useful to consider diabetes duration in order to stratify cardiovascular risk of type 2 diabetic patients and there was significant difference for age at onset of DM for T2DM without CVDs groups; for T2DM with CVDs groups; (P<0.05). This explained our result about T2DM with CVDs had older age because T2DM onset on older age and older adults with diabetes are at substantial risk for both acute and chronic micro vascular and cardiovascular complications of the disease.

O'Keefe *et al.*, (2011); Wannamethee *et al.*, (2011); illustrated that both early and late onset of diabetes are associated with increased risk of major coronary heart disease events and mortality, but only early onset of diabetes appears to be a CHD equivalent. This result was compatible with the study that shown association between age at onset of DM and CVD development. Based on our result, there was positive relation between diabetes duration and CVD complication in type 2 diabetic patients.

3. Cardiac biomarkers levels among the studied groups

Table (3) shows serum cardiac enzyme activities in the three studied groups including lactate dehydrogenate (LDH), creatine kinase (CK) and creatine kinase muscle brain (CKMB). The mean of LDH were 328.8 (210 - 476), 363.5 ± 69.6 (240 - 592) and 467.4 ± 212.6 (261 - 1146) U/L among group 1, group 2 and group 3, respectively. ANOVA test showed significant difference in the level of serum LDH among three groups (P<0.001), Post- hoc test revealed significant difference between group 2 within group 3, and group 1 within group 3 (P<0.001). In contrast, no significant differences were found between group 1 and group 2.

However, On the other hand, the mean of CK and CKMB

was gradual increased with values of 101.5 ± 39 (36 - 175), 124 ± 63.6 (47 - 290) and 208.8 ± 312.5 (57 - 1695) U/L for CK and 13.1 ± 4.3 (47 - 290), 20.8 ± 10.2 (9 - 61) and 34.4 ± 50.4 (10 - 280) for CKMB among Gr1, Gr 2 and Gr 3, respectively. The ANOVA test showed significant difference in the mean level of serum CK among three groups (P <0.001) and Post-hoc test showed significant difference between group 1 and group 3 (P <0.001), Gr 1 and Gr 3

Table Error! No text of specified style in document.**(2):** Duration of diabetes in patient groups

Variables	Group 2 (T2DM)	Group3 (T2DM&CVD)	t	P-value
Diabetic duration (years) Range	6.7 ± 4.9 [c] 1 – 24	11 ± 9.2 1 – 40	-3.193	0.000
Age at onset of DM (years) Range	47.9 ± 7.1[c] 24 – 61	49.7 ± 9.5 22 - 64	-1.176	0.040
Age at onset of CVDs (years) Range	-	53.5 ± 5.9 39 - 63	-	-

[a ,b, c]: significant at p≤0.05

Obviously, cardiac enzyme activities increase gradually among group 1, group 2 and group 3, respectively. This increase was clinical and statistically significant for group 3 compare with group 1 and group 2 cardiac enzyme activities increase gradually among three studied groups respectively. This increase was clinical and statistically significant for group three compare with group 2 and group 1. ANOVA test showed significant difference in the level of serum LDH among three groups (*P*<0.001), Post- hoc test revealed significant difference between group 2 and 3, group 1 and 3 (*P*<0.001). In contrast, no significant differences were found between group 1 and group 2. Also ANOVA test showed significant difference in the mean level of serum CK and CKMB among three groups (*P* <0.001) and Post-hoc test showed significant difference between group one with group two and three *(P* <0.001).Diabetes is an independent risk factor for cardiovascular disease. Cardiac enzymes have been demonstrated to be important prognostic determinants to identify high risk patients (Gencer *et al*., 2018).

The present study, showed that the increase in serum LDH, CK and CKMB activities in the T2DM with CVDs group was significant compared to the T2DM without CVDs and control groups. Similar results were previously reported by Davey and Atlee, (2011); Ali *et al*., (2016). The significant elevation of LDH, CK and CKMB may be explained by their specificity to CVDs diagnostic.

Table (3) showed serum NT-BNP mean 157.3 (130.9–184.5), 72.9 ± 45.2 (21.7 - 181.9) and 334.3 ± 121.8 (73.6 - 593.1) pg/ml among group 1, group 2 and group 3, respectively. ANOVA test showed significant difference in the level of serum NT-BNP among three groups (*P*<0.001). Post-hoc test was clarified a significant difference between group 1 with group 2 and group 3 (*P*<0.001). There were no significant difference was found between group 2 and group 3.This indicated that NT-BNP predict biomarkers for heart disease in T2DM patients. This results are in agreement with Santaguida *et al*., (2014) found that NT-BNP as independent predictors of mortality, morbidity, or combined mortality and morbidity outcomes in persons with acute decompensated heart failure and Wang *et al.*, (2017) provide additional prognostic information to NT-proBNP in the population the peptides are regarded as equal for the diagnosis of both acute and chronic heart failure. In the same time Peleg *et al*., (2013) clarified that NT-BNP secretion depends on myocardial wall stress, hence the role of blood BNP as a marker of heart failure. NT-BNP level may predict the outcome of acute coronary syndrome via a heart failure mechanism, but regarding (Mitchell *et al*., 2015) found there was associated with NT-BNP level in this cross-sectional study of asymptomatic adults free of overt coronary artery disease, suggesting that higher NT-proBNP levels may reflect subclinical myocardial microvascular dysfunction .Also the results of the study coincides with Görmüs *et al.*, (2010) that found NT-BNP levels known to be elevated in T2DM patients with asymptomatic diastolic

dysfunction. It has been acknowledged that subjects with higher NT-BNP levels are more likely to have conventional cardiovascular risk factors.

These findings have important clinical implications because patients with T2DM have an increased risk of developing CVDs. Identifying novel risk factors for CVDs may help in the development of strategies for the prevention and treatment of CVDs in T2DM patients.

Table (3) Cardiac biomarkers levels among the studied groups

Biomarkers	Group 1 (Control)	Group 2 (T2DM)	Group 3 (T2DM &CVD)	*P*-value
LDH Range	328.8 ± 62.4[b] 210 - 476	363.5 ± 69.6[c] 240 - 592	467.4 ± 212.6 261 - 1146	0.000**
CK Range	101.5 ± 39[a b] 36 - 175	124 ±63.6 47 - 290	208.8 ± 312.557 - 1695	0.000**
CKMB Range	13.1 ± 4.3[a b] 4 - 20	20.8 ± 10.2 9 - 61	34.4 ± 50.4 10 - 280	0.000**
NT-BNP (pg/ml) Range	72.9 ± 45.2[a b] 21.7 - 181.9	334.3 ± 121.8 73.6 - 593.1	306.2 ± 100.6 138.2 - 553.5	0.000**

[a ,b, c]: significant at p≤0.05)

Conclusions

*Many risk factors like BMI, smoking and hypertension have a role in the complications of diabetic and cardiac patients.

*Assessment of diabetic patients is challenging in clinical practice resulting frequently in a typical presentation of signs and symptoms, as wells as under recognizing cardiovascular risk. Cardiac biomarkers: LDH,CK,CKMB and NT-BNP, hence improved prediction of cardiac mortality incidences in T2DM are essential.

*More clinical data are required to understand the critical numerous combinations of markers that are involved in increasing risk stratification.

*The predictive role of novel cardiac biomarkers for diabetics in primary prevention settings requires much more additional studies and clinical strategies must be followed in patients T2DM to avoid an increased risk of developing CVDs.

References

Ali, F., Naqvi, S. A. S., Bismillah, M., and Wajid, N. (2016). Comparative analysis of biochemical parameters in diabetic and non-diabetic acute myocardial infarction patients. *Indian Heart Journal*, 68(3), 325-331.

Annual report chronic disease/ Gaza strip, (2017). Access on 15/12/2018. www.mezan.org/uploads/files/8532.pdf.

Berezin, A. E. (2017). Cardiac biomarkers in diabetes mellitus: new dawn for risk stratification?. Diabetes and Metabolic Syndrome: *Clinical Research & Reviews*, 11, S201-S208.

Bodor, G. S. (2016). Biochemical markers of myocardial damage. *Ejifcc*, 27(2), 95.

Davey M. S., and Atlee C. W. (2011). Inotropic and cardio protective effect of *Terminalla paniculata* Roth bark extract in doxorubicin induced cardio toxicity in rats. *International Journal of Research in Ayurveda and Pharmacy*, 2(3): 869-875.

Gencer, B., Rigamonti, F., Nanchen, D., Klingenberg, R., Räber, L., Moutzouri, E. and Lüscher, T. F. (2018). Prognostic values of fasting hyperglycemia in non-diabetic patients with acute coronary syndrome: A prospective cohort study. *European Heart Journal*: *Acute Cardiovascular Care*, *7*(8),

Gidding, S. S., Bacha, F., Bjornstad, P., Katz, L. E. L., Levitsky, L. L., Lynch, J. and Haymond, M. (2018).

Cardiac biomarkers in Youth with Type 2 diabetes mellitus: results from the TODAY Study. *The Journal of pediatrics* 192, 86-92.

Gimeno Orna J.A., Blasco Lamarca Y., Campos Gutierrez B., Molinero Herguedas E., Lou Arnal L. M. (2014): Morbidity and mortality cardiovascular risk in dependence of type 2 diabetes duration. *Clin Investig Arterioscler: publicacion oficial de la Sociedad Espanola de Arteriosclerosis*, 26(3): 122-130.

Goetze J. P., Zois N. E. (2016). Cardiac natriuretic peptides in plasma: from prediction to precision medicine. *Lancet Diabetes Endocrinol*. (10): 803-5.

Görmüs¸ U, Ozmen D, Ozmen B, et al (2010): Serum Nterminal- pro-brain natriuretic peptide (NT-pro-BNP) and homocysteine levels in type 2 diabetic patients with asymptomatic left ventricular diastolic dysfunction. *Diabetes Res Clin. Pract* 87: 51-56.

Martín-Timón, I., Sevillano-Collantes, C., Segura-Galindo, A., & del Cañizo-Gómez, F. J. (2014). Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World Journal of Diabetes*, 5(4), 444.

Mitchell, A., Misialek, J. R., Folsom, A. R., Duprez, D., Alonso, A., Jerosch-Herold, M., and Konety, S. H. (2015). Usefulness of N-terminal Pro–brain Natriuretic Peptide and Myocardial Perfusion in Asymptomatic Adults (from the Multi-Ethnic Study of Atherosclerosis). *The American journal of Cardiology*, 115(10), 1341-1345.

O'Keefe, J. H., Abuannadi, M., Lavie, C. J., & Bell, D. S. (2011, February). Strategies for optimizing glycemic control and cardiovascular prognosis in patients with type 2 diabetes mellitus. In Mayo clinic proceedings (Vol. 86, No. 2, pp. 128-138). Elsevier.

Palestine National Diabetes Program, WDF15-1304 (2016). West Bank, Palestine. Access on 15/12/2018. https://www.worlddiabetesfoundation.org/projects/west-bank-and-gaza-wdf15-1304

Patel, P. M. (2014). The evaluation of cardiac markers in diabetic and non diabetic patients with myocardial infarction. *Age* (years), 52, 8-02.

Peleg, A., Ghanim, D., Vered, S., & Hasin, Y. (2013). Serum corin is reduced and predicts adverse outcome in non-ST-elevation acute coronary syndrome. *European Heart Journal: Acute Cardiovascular Care*, 2(2), 159-165.

Sanchez OA, Duprez DA, Bahrami H, Peralta CA, Daniels LB, Lima JA, et al. Changes in N-terminal pro-B-typ e natriuretic peptide and incidence of diabetes: The Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Metab*. 2015; 41(5):378-86.

Santaguida, P. L., Don-Wauchope, A. C., Oremus, M., McKelvie, R., Ali, U., Hill, S. A., and Sohel, N. (2014).

BNP and NT-proBNP as prognostic markers in persons with acute decompensated heart failure: a systematic review. *Heart FailureR*, 19(4), 453-470.

Seuring T, Archangelidi O, Suhrcke M. (2015).The economic costs of type 2 diabetes: a global systematic review. *Pharmacoeconomics*. ;33: 811–31.

Wang, J., Tan, G. J., Han, L. N., Bai, Y. Y., He, M., & Liu, H. B. (2017). Novel biomarkers for cardiovascular risk prediction. Journal of *Ggeriatric Cardiology: JGC*, 14(2), 135.

Wannamethee S. G., Shaper A.G., Whincup P.H., Lennon L., & Sattar N. (2011): Impact of Diabetes on Cardiovascular Disease Risk and All-Cause Mortality in Older Men: Influence of Age at Onset, Diabetes Duration, and Established and Novel Risk Factors. *Arch Intern M*ed., 171(5): 404-410.5.4

World Health Organization. (2016). Global report on

diabetes. World Health Organization.