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# Assessment of Inflammatory Markers; Il-8 And TNF-α As Predictors of Atherosclerotic Progression of Coronary Arteries in Egyptian Patients With or Without Diabetes

KEYWORDS: Coronary Artery Disease, Interleukin-8, Tumor Necrosis Factor-alpha.

| Nearmeen M. Rashad | Alaa A. Farag | Hassan M. Hassanin |
|---|---|---|
| Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt | Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt | Internal Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt |

| Amal S. El-Shal | Amer A. Ateya. |
|---|---|
| Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt | Cardiology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt |

**ABSTRACT** *Background: Cardiovascular complications are the leading cause of morbidity among patients with diabetes, and ischemic heart disease is the most common cause of death. Several inflammatory mediators have been proposed to contribute to the pathogenesis of atherosclerosis. The aim of this study was to evaluate the levels of interleukin -8 (IL-8) and tumor necrosis factor-alpha (TNF-α) in Egyptian patients with coronary artery disease. Moreover, we aimed to clarify the possible relationships of IL-8, TNF α , diabetes, as well as other cardiovascular risk factors in Egyptian cardiac patients with different degrees of coronary artery occlusion.*

*Methods: One hundred healthy control subjects, age ,sex and smoking habits were matched with patients (n=100). A retrospective analysis was conducted on patients with coronary artery disease ( CAD), who underwent coronary angiography at cardiology department of Zagazig University Hospitals. The enrolled patients were divided into two groups : diabetic cardiac patients (n=45) and non-diabetic cardiac patients (n=55). Both diabetic and non-diabetic cardiac patients were further subdivided into four groups according to number of vessel occlusion. IL-8 and TNF-α were measured using immune-enzymatic ELISA kits.*

*RESULTS: Diabetic patients with CAD had significantly high values of clinical and biochemical parameters of metabolic syndrome. Also, IL-8 and TNF α levels were significantly higher in diabetic cardiac patients compared to non-diabetic ones. Moreover, when we stratified cardiac patients according to coronary artery occlusion we found that patients with three vessel occlusion had higher levels of inflammatory markers; IL-8 and TNF α, and for further evaluation, IL-8 and TNF α levels in cardiac patients were independently correlated with LDL, fasting blood glucose, waist / hip ratio ,fasting insulin, HbA1c and HOMA-IR by linear regression analysis.*

*Conclusion: IL-8 and TNF-α levels of cardiac patients were associated with diabetes, and may be used as surrogate markers of CAD in diabetes and as strong predictive factors for progression of atherosclerosis in the coronary arteries.*

**Introduction**

The prevalence of diabetes mellitus (DM) is rapidly increasing worldwide, due to the increase in type 2 DM which represents more than 90% of all cases of diabetes [1,2]. Cardiovascular complications are the leading cause of morbidity among patients with diabetes, and ischemic heart disease is the most common cause of death [3,4]. The risk for cardiovascular disease (CVD) is two to eight-folds higher in patients with diabetes compared to non-diabetic individuals of similar age, sex, and ethnicity [5-7]. Moreover, among patients with CAD, diabetes is associated with an increased risk of developing acute coronary syndrome and an increased risk of death after an acute myocardial infarction [8,9].

Many studies confirmed the association between inflammations triggers factors and occurrence of CAD [10]. In CAD, lipids accumulate in the intima of coronary arteries which is associated with mononuclear cell infiltration and smooth muscle proliferation [11]. Cytokines play major role in activation of adhesion molecules and chemokine expression, involved in leukocyte recruitment [12]. Activation of pro-inflammatory cytokines including interleukin-1 (IL- 1) and TNF-α and initiation of an immune mediated response from the site of plaque formation in arterial wall makes a complex of reactions with a number of immune component being involved in atherosclerosis[13].

Innate immune responses beside adaptive immunity have major role in the initiation of atherosclerosis [14]. Infiltrating monocytes and macrophages play major role in pro-inflammatory cytokine productions in atherosclerosis. Some investigators have proposed that TNF-α, IL-2 and IL-10 may be potential markers in the prediction of independent risk factors for future myocardial infarction [15].

Endothelial cells (ECs) and smooth muscle cells (SMCs) can both contribute and respond to cytokine production, thus promoting atherosclerosis. Recently, we demonstrated; using a novel in vitro EC/SMC co-culture hemodynamic flow system that the human chemokine interleukin-8 (IL-8/CXCL8) is secreted at higher levels by ECs exposed to an atheroprone flow environment, compared to atheroprotective flow [16]. Also, atheroprone flow caused ECs/SMCs to undergo inflammatory priming, whereby the adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1), was upregulated in both cell types compared to atheroprotective flow [16].

The fact that IL-8 is implicated in atherogenesis is well established [17]. Macrophages and foam cells from human atherosclerotic lesions have been shown to produce IL-8, and macrophages from advanced lesions in mice strongly express CXCR2 [19]. Furthermore, SMCs of atherosclerotic lesions proliferate and migrate to the intima of the vessel wall in response to IL-8 [20,21].

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In addition to in vitro studies, increased serum levels of IL-8 have been associated with CAD and adverse outcome after acute myocardial infarction [22-24]. The American Diabetes Association (ADA) recommends testing of diabetic patients with the following characteristics for cardio vascular risk: typical or atypical cardiac symptoms, resting ECGs suggestive of ischemia or infarction, peripheral or carotid occlusive arterial disease, a sedentary lifestyle, over 35 years old, plan to begin a vigorous exercise program, or show traditional and diabetes-specific risk factors (dyslipidaemia , hypertension, smoking, or with family history of premature CAD, micro/macroalbuminuria) [25].

TNF-α is a mediator of the acute phase response and is involved in production of other inflammatory mediators including chemokines with important role in recruitment of leucocytes to the site of inflammation, and so, augmented risk of thromboembolic complications [26].

415 million people have diabetes in the world and more than 35.4 million people in the MENA Region (the Middle East and North Africa region) and by 2040 this will rise to 72.1 million. There were over 7.8 million cases of diabetes in Egypt in 2015(IDF, 2015). In our Egyptian population, cardiovascular complications of diabetes are the leading cause of morbidity and mortality.

Therefore, the purpose of current study is to investigate serum concentration of IL-8 and TNF-α in Egyptian patients with coronary artery disease. Moreover, we aimed to clarify the possible relationships of IL-8, TNF – , diabetes, as well as other cardiovascular risk factors in Egyptian cardiac patients with different degrees of coronary artery occlusion.

**Subjects and methods**

Our study includes one hundred healthy control subjects, age, sex and smoking habits were matched with patients (n=100). A retrospective analysis was conducted on patients with CAD, who underwent coronary angiography at cardiology department of Zagazig University Hospitals. The enrolled patients were divided into two groups: diabetic cardiac patients (n=45) and non-diabetic cardiac patients (n=55). The diagnosis of DM was based on fasting plasma glucose levels of ≥126 mg/dL (7.0 mmol/L) or 2-h postprandial plasma glucose levels of ≥200 mg/dL (11.1 mmol/L). Both groups were further subdivided into four groups according to number of vessel occlusion.

The decision to perform coronary angiography was based on symptoms consistent with the diagnosis, an abnormal electrocardiogram (ECG), positive findings in standard exercise tests, or abnormal findings in radio nuclear studies. All patients were subjected to thorough history taking and full clinical assessment including blood pressure. Height, waist circumference, and hip circumference were measured to calculate obesity indices. Anthropometric variables including body mass index (BMI) calculated as weight in kg/ height in (meters) $^2$ and waist circumference (cm)/hip circumference (cm) (WHR) were measured.

Patients suffering from any acute infection, acute renal failure, any endocrine disorder except diabetes mellitus, patients with thrombocytopenia, liver disease, cardiogenic shock, acute myocardial infarction within 48 hour, unstable CAD,variant angina, history of coronary artery bypass graft surgery, prior revascularization, malignancy , autoimmune disease , or recent infectious disease were excluded from the study. The ethical committee of Faculty of Medicine, Zagazig University approved our study protocol, and all participants assigned written informed consent.

**Blood sampling**

Blood samples were drawn from all subjects after an overnight fasting and divided into 3 portions and HbA1c; 1 ml of whole blood was collected into evacuated tubes containing fluoride for fasting blood glucose. Serum was separated immediately from remaining part of the sample and stored at 20 °C until analysis.

**Biochemical measurements**

We determined fasting blood glucose using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol and triglycerides were measured by routine enzymatic methods (Spinreact, Girona, Spain). HDL cholesterol was determined after precipitation of the apoB-containing lipoproteins. LDL cholesterol was calculated using the Friedewald formula (12).

**Cytokine assays**

Serum level of cytokines including, IL-8 and TNF–α was determined by ELISA (enzyme-linked immunosorbent assay) used commercially available kits purchased from Bender Medsystems (Austria).

**Statistical analysis**

Statistical analysis was performed using the Statistical Package for the Social Sciences for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed. One-way analysis of variance (ANOVA) test was done to compare different parameters between more than two groups. Pearson correlation coefficient was used to assess the association between IL-8 and TNF α, clinical, biochemical tests and other studied metabolic parameters in patients with coronary artery disease. A linear regression analysis was performed to detect the main predictors of IL-8 and TNF α levels in coronary artery disease. P-values were considered significant if <0.05.

**Results**

Among case individuals,58% were male and 42% female, and in control individuals 60% male and 40% female. The mean age of case group was (40.56±7.48 years ) and in controls (41.96±5.75 years). The case and control individuals were thus balanced in terms of age and sex . Clinical and anthropometric characteristics of the studied groups are summarized in Table 1.

There were significant differences between case and control group as regard systolic BP, diastolic BP, BMI, Waist-hip ratio, total cholesterol ,TG ,LDL.c ,HDL.c ,fasting blood glucose, fasting insulin, HbA1c and HOMA-IR (P □ 0.05). Moreover , diabetic patients with coronary artery disease had significantly higher values of systolic BP, diastolic BP, BMI, Waist-hip ratio, total cholesterol ,TG ,LDL.c , fasting blood glucose, fasting insulin, HbA1c and HOMA-IR. On the other hand diabetic patients with coronary artery disease had significantly lower values of HDL.c compared with other groups ( table 1).

**Table 1: Clinical, anthropometric and biochemical characteristics of the studied groups**

| Characteristics | Control (n=100) | Non-diabetic cardiac patients (n=55) | diabetic cardiac patients (n=45) |
|---|---|---|---|
| BMI(kg/m$^2$) | 22.4±1.29* | 32.0±5.23 | 31.57±4.97* |

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| | | | |
|---|---|---|---|
| Waist/hip ratio | 0.80±0.046 | 1.14± 0.18 | 1.13±0.177* |
| SBP (mmHg) | 117.7±3.9 | 129.2± 11.17935 | 130.9±12.7* |
| DBP (mmHg) | 75.8±4.12678 | 83.3±11.17 | 84.3±10.20* |
| T-Cho (mg/dl) | 182.6±17.22* | 214.27±29.35* | 207.08±33.53* |
| TG (mg/dl) | 179.4±14.60* | 229 ±33.739* | 228.3±41.92* |
| LDL.c (mg/dl) | 100.3±20.29 | 131.45±27.55 | 124.8±34.015* |
| HDL.c (mg/dl) | 46.4±6.154 | 37.01±5.233 | 36.5±4.97* |
| FBG (mg/dl) | 88.35±4.026* | 83.18±14.46* | 188.6±31.28* |
| F. Insulin(µU/dl) | 7.58±2.02* | 12.30±2.139* | 27.91± 4.627* |
| HbA1c (%) | 5.69±0.517* | 5.34±0.91* | 11.25±1.86* |
| HOMA-IR | 1.66±0.470* | 2.60± 0 .90* | 13.3±4.418* |

BMI, body mass index; FBS, fasting blood glucose; HbA1c, hemoglobin A1c; Total-C, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol, HOMA-IR homeostasis model assessments of insulin resistance,

There were significant differences between cardiac non diabetic patients when stratified according to number of coronary occlusion as regard systolic BP, diastolic BP, total cholesterol ,TG ,Ld. ,HDL .c, fasting blood glucose, fasting insulin, HbA1c and HOMA-IR (P > 0.05). Moreover , non-diabetic patients with three vessel occlusion had significantly higher values of systolic BP, diastolic BP, BMI, Waist-hip ratio, total cholesterol ,TG ,LDL .c, fasting blood glucose, fasting insulin, HbA1c and HOMA-IR. On the other hand, there were no significant differences as regard BMI, waist/hip ratio and HDL ( table 2).

**Table 2: Clinical, anthropometric and biochemical characteristics of non-diabetic cardiac patients stratified according to number of vessel occlusion by coronary angiography**

| Characteristics | Non-diabetic patients (n =55) | | | |
|---|---|---|---|---|
| | No vessel(**n** =13) | One vessel(**n** =11) | Two vessel(**n** =10) | Three vessel(**n** =21) |
| BMI(kg/m$^2$) | 29.83±4.04* | 35.72±2.4* | 30.8±5.202 | 31.6±6.110 |
| Waist/hip ratio | 1.06±0.144* | 1.27±0.08* | 1.1±0.185 | 1.13±0.218 |
| SBP (mmHg) | 122±7.24 | 119.8±**22**.4 | 131.4±8.66* | 137.61±10.6* |
| DBP (mmHg) | 73.08±12.11 | 81.72±8.77 | 82.20±9.80* | 90.52±7.400* |
| T-Cho (mg/dl) | 185.4±17.242 | 191.81±5.6 | 212±19.32* | 244.5±14.82* |
| TG(mg/dl) | 209.16±32.87 | 210±44.497 | 247.5±33.35* | 243.3±9.53* |
| LDLc (mg/dl) | 108.75±17.06 | 109.1±10.6 | 126.7±21.64* | 159.19±14.3* |
| HDLc(mg/dl) | 34.8±4.041 | 40.72±2.4* | 35.8±5.202 | 36.6±6.110 |
| FBG (mg/dl) | 68.84±8.107 | 71.85±2.63 | 81.34±9.081* | 96.62±6.96* |
| F. Insulin(µU/dl) | 10.18±1.199 | 10.62±0.38 | 12.03±1.343* | 14.29±1.03* |
| HbA1c (%) | 4.43±**0**.521 | 4.62±0.169 | 5.23±**0**.584* | 6.218±1.448* |
| HOMA-IR | 1.75±0.476 | 1.88±0.135 | 2.44±0.526* | 3.42±0.494* |

BMI, body mass index; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; Total-C, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HOMA-IR homeostasis model assessments of insulin resistance

When cardiac diabetic patients were stratified according to number of coronary occlusion, diabetic patients with three vessel occlusion had significantly higher values of systolic BP, diastolic BP, BMI, Waist-hip ratio, total cholesterol ,TG ,LDL .c ,HDL .c ,fasting blood glucose, fasting insulin, HbA1c and HOMA-IR (P □ 0.05) .Moreover ,there were significant differences among diabetic patients with coronary artery disease as regard BMI, Waist-hip ratio, fasting blood glucose, fasting insulin, HbA1c and HOMA-IR. On the other hand, there were no significant differences as regard other parameters ( table 3).

**Table3: Clinical, anthropometric and biochemical characteristics of diabetic cardiac patients stratified according to number of vessels occlusion by coronary angiography**

| Characteristics | Diabetic patients (n =46) | | | |
|---|---|---|---|---|
| | No vessel **(n** =7) | One vessel **(n** =12) | Two vessels **(n** =12) | Three vessels**(n** =14) |
| BMI(kg/m$^2$) | 33±3.464* | 33.3±4.35* | 32.91±3.2* | 29±6.36* |
| Waist/hip ratio | 1.17±0.123* | 1.19±0.155* | 1.175±**0**.114* | 1.03±0.227* |
| SBP (mmHg) | 119.7± 0.487 | 123.5±8.35 | 128.25±9.92 | 144.14± 9.8* |
| DBP (mmHg) | 81.28±12.188 | 77.5±7.179 | 84.25±10.437 | 91.07±7.054* |
| T-Cho (mg/dl) | 173.8±19.67 | 187.08±12.87 | 201±23.6* | 247.85±11.5* |
| TG(mg/dl) | 242.85±49. 23 | 197±11.38* | 243±57.039 | 237±21.2* |
| LDLc (mg/dl) | 87.28±24.615* | 109.25±13.72 | 114.48±20.01 | 166.45±13.08* |
| HDLc(mg/dl) | 38±3.464 | 38.33±4.35 | 37.91±3.203 | 34.±6.36* |
| FBG (mg/dl) | 242.85±49.2* | 197.5±11.381* | 243±57.03* | 237.±21.209* |
| F. Insulin(µU/dl) | 23.64±2.676* | 24.6±2.921* | 27.34±3.21* | 32.8±3.721* |
| HbA1c (%) | 9.5±1.079* | 9.94±1.178* | 11.02±1.294* | 13.26±1.5* |
| HOMA-IR | 9.437±2.03* | 10.28±2.22* | 12.63±3.02* | 18.27±3.566* |

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In patients with coronary artery disease, TNF-α was positively correlated with systolic BP, diastolic BP, total cholesterol, LDL .c, fasting blood glucose, fasting insulin, HbA1c and HOMA-IR. On the contrary, there was non-significant correlation between TNF-α and other clinical and biochemical characters .As regard IL-8,in patients with coronary artery disease, IL-8 was positively correlated with systolic BP, diastolic BP, total cholesterol ,LDL .c ,fasting blood glucose, fasting insulin, HbA1c and HOMA-IR. On the contrary, there was non-significant correlation between IL-8 and other clinical and biochemical characters.

Table 4 Pearson's correlation coefficient between TNF-α (pg/ml), Interleukin-8 (ng/ml), clinical, anthropometric and biochemical characteristics in cardiac patients (n = 100)

| | TNF-α | | **Interleukin-8** | |
|---|---|---|---|---|
| **characteristics** | r | p | r | p |
| BMI(kg/m$^2$) | 0.103 | NS | 0.110- | NS |
| Waist/hip ratio | -.103 | NS | 0.110 | NS |
| SBP (mmHg) | .503 | 0.001< | .540 | 0.001< |
| DBP (mmHg) | .326 | 0.001< | .351 | 0.001< |
| T-Cho (mg/dl) | .491 | 0.001< | .547 | 0.001< |
| TG (mg/dl) | .169 | NS | .186 | NS |
| LDL.c (mg/dl) | .478 | 0.001< | .533 | 0.001< |
| HDL.c (mg/dl) | -.103 | NS | -.110 | NS |
| FBG (mg/dl) | .961 | 0.001< | .942 | 0.001< |
| F. Insulin(µU/dl) | .961 | 0.001< | .942 | 0.001< |
| HbA1c (%) | .970 | 0.001< | .952 | 0.001< |
| HOMA-IR | .966 | 0.001< | .951 | 0.001< |

Linear regression analyses in cardiac patients to test the influence of the main independent variables against serum TNF α levels (dependent variable).

In cardiac patients (n=100), linear regression analysis showed that, TNF α levels were independently correlated with LDL .c, fasting blood glucose, waist /hip ratio , fasting insulin, HbA1c and HOMA-IR (P < 0.001) (**Table 5**).

table 5. linear regression analysis in Non-diabetic CAD patients and Diabetic CAD individuals to test the influence of the main independent variables against TNF-α (pg/ml) (dependent variable).

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | 95.0% C I | |
|---|---|---|---|---|---|---|---|---|
| B | | Std. Error | Beta | | | Lower Bound | Upper Bound | |
| 1 | (Constant) | 1.530 | .058 | | 26.204 | .000 | 1.414 | 1.646 |
| | Age | -.001- | .000 | -.003- | -1.425- | .157 | -.002- | .000 |
| | SBP (mmHg) | -.001- | .000 | -.005- | -1.870- | .065 | -.002- | .000 |
| | DBP (mmHg) | .001 | .001 | .003 | .944 | .348 | -.001- | .002 |
| | LDLc (mg/dl) | -.001- | .000 | -.022- | -4.360- | .000 | -.002- | -.001- |
| | TG(mg/dl) | .000 | .000 | -.006- | -2.451 | .016 | -.001- | .000 |
| | FBG (mg/dl) | -.266- | .004 | -7.921- | -62.9- | .000 | -.274- | -.258- |
| | HbA1c (%) | 5.064 | .074 | 8.565 | 68.197 | .000 | 4.917 | 5.212 |
| | HOMA-IR | .105 | .003 | .333 | 34.053 | .000 | .099 | .111 |
| | Waist/hip ratio | -.073- | .019 | -.007- | -3.846- | .000 | -.110- | -.035- |
| | | | | | | | | |

**Linear regression analyses in cardiac patients to test the influence of the main independent variables against serum interleukin -8 levels (dependent variable).**

In cardiac patients (n=100), linear regression analysis showed that, IL-8 levels were independently correlated with LDL .c, TG, fasting blood glucose, waist /hip ratio ,fasting insulin, HbA1c and HOMA-IR (P < 0.001) (**Table6**).

**Table 6.** Linear regression analysis in non-diabetic CAD patients and diabetic CAD individuals to test the influence of the main independent variables against interleukin -8(ng/ml) (dependent variable).

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. | 95.0% C.I | |
|---|---|---|---|---|---|---|---|---|
| B | | Std. Error | Beta | | | Lower Bound | Upper Bound | |
| 1 | (Constant) | .285 | .063 | | 4.485 | .000 | .159 | .411 |
| | Age | -.001- | .001 | -.004- | -1.349- | .181 | -.002- | .000 |
| | SBP (mmHg) | -.001- | .001 | -.007- | -2.121- | .037 | -.002- | .000 |
| | DBP (mmHg) | .001 | .001 | .003 | .867 | .388 | -.001- | .002 |
| | LDLc (mg/dl) | .003 | .000 | .059 | 9.849 | .000 | .003 | .004 |
| | TG(mg/dl) | .001 | .000 | .014 | 4.956 | .000 | .000 | .001 |
| | FBG (mg/dl) | -.230- | .005 | -7.421- | -50.022- | .000 | -.239- | -.221- |
| | HbA1c (%) | 4.326 | .081 | 7.936 | 53.607 | .000 | 4.166 | 4.487 |
| | HOMA-IR | .123 | .003 | .424 | 36.747 | .000 | .116 | .129 |
| | Waist/hip ratio | .060 | .021 | .006 | 2.943 | .004 | .020 | .101 |
| | | | | | | | | |

Comparison of TNF α level of the studied groups (Fig.1 ,3and 5):

Diabetic patients with coronary artery disease had significantly higher values of **TNF** α (9.32±1.546) more than non-diabetic patients with coronary artery disease **(6.3±0.863)** and controls **(1.8491±0.174).** Moreover, when non-diabetic patients with coronary artery disease were stratified according to number of coronary artery occlusion to four group; no vessel (n=13) ,( **5.45±0.507**), single vessel (n=11) , (**5.64±0.164**) two vessel (n=10) ,( **6.23±0.568)** and three vessel (n=21)**,( 7.19±0.436),** there was high significant difference among these groups. Also, when diabetic patients with coronary artery disease were stratified according to number of coronary artery occlusion to four group; no vessel (n=7) ,( **7.9±0.894**), single vessel (n=12) , (**8.2±0.976**) two vessel (n=12) **,( 9.13±1.072)** and three vessel (n=14**),( 10.99±1.243**), there was high significant difference among these groups.

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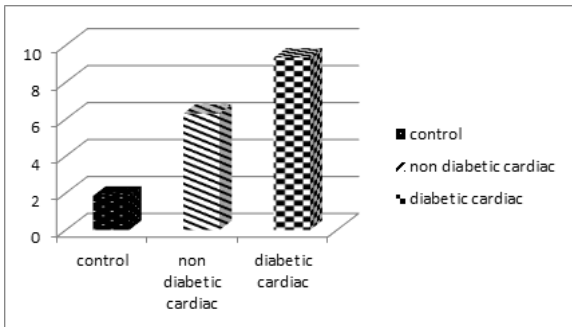


**Fig 1: TNFα in studied patients**

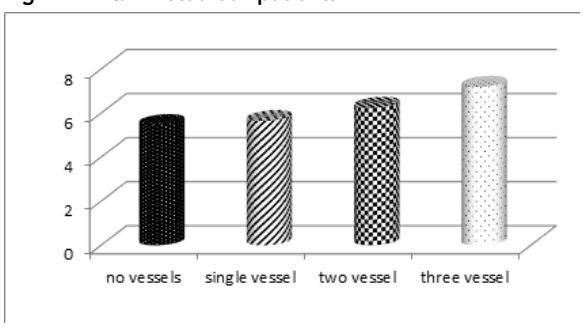


**Fig 3:TNFα in cadiac non- diabetic patients stratified according to number of vessel occlusion**

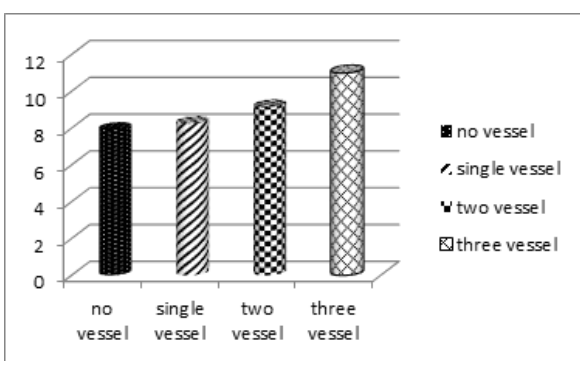


**Fig 5: TNFα in cadiac diabetic patients stratified according to number of vessel occlusion**

Comparison of interleukin-8 level of the studied groups (Fig.2,4 and 6):

Diabetic patients with coronary artery disease had significantly higher values of IL-8 **(7.79±1.53)** more than non-diabetic patient with coronary artery disease **(5.1571±0.863)** and controls (**1.5± 0.174).** Moreover ,when non-diabetic patients coronary artery disease were stratified according to number of coronary artery occlusion to four group; no vessel (n=13) ,( **4.30±0.507**), single vessel (n=11) , (**4.49±0.164**) two vessel (n=10) ,( **5.09±0.568)** and three vessel (n=21**) ,( 6.04±0.436),** there was high significant difference among these groups. Also, when diabetic patients coronary artery disease were stratified according to number of coronary artery occlusion to four group; no vessel (n=7) ,( **6.36±0.894**), single vessel (n=12) , (**6.7±0.886**) two vessel (n=12) **,( 7.59±1.072)** and three vessel (n=14),**( 9.47±1.148),** there was high significant difference among these groups.

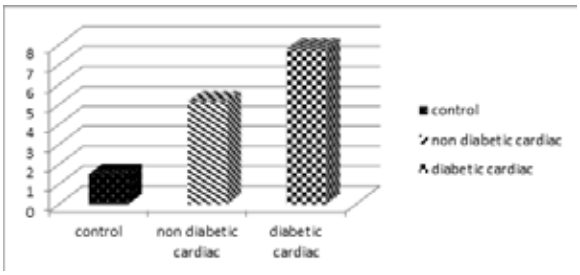


**Fig.2:interleukin-8 in studied groups**

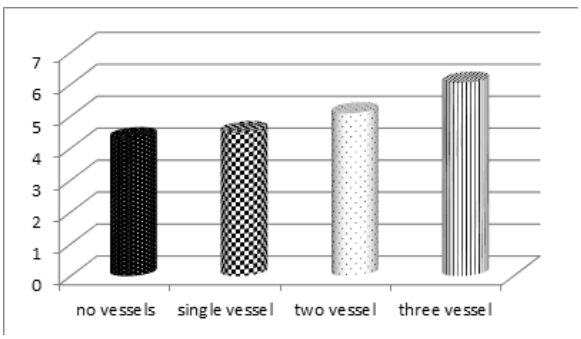


**Fig 4: interleukin-8 in cadiac non- diabetic patients stratified according to number of vessel occlusion**

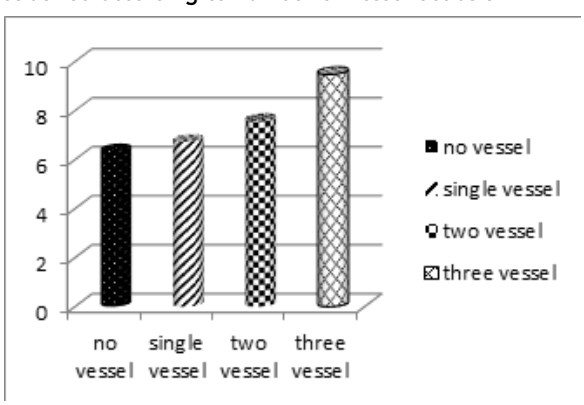


**Fig 6: interleukin-8 in cadiac diabetic patients stratified according to number of vessel occlusion**

**Discussion**

Cardiovascular diseases (CVDs) account for about 38% of all deaths worldwide. The main cause of CVD is Coronary Artery Disease (CAD), the leading cause of the death in developed countries [27]. By 2015, almost 20 million people will die from CVDs [28]. In Egypt, CVDs are now the main causes of death among Egyptians. In 1970, CVDs had accounted for 12.4% of all deaths, whereas two decades later they were responsible for 42.5% of the mortality [29].

The prevalence, incidence, and mortality of all forms of CVD were strikingly higher for people with diabetes than those without diabetes [31-32]. According to the World Health Organization, the prevalence of CVD in diabetic patients ranges from 26 to 36% [11].

Major risk factors of CVDs include hypercholesterolemia (HC), hypertension (HT), diabetes Mellitus (DM) and smoking [30]. CAD is mainly caused by an interaction between genetic and environmental factors [34]. A family history

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of premature CAD, DM, cigarette smoking, hypertension, hyperlipidemia, atherosclerosis, obesity, and high-fat/low-fiber diets have been established as a contributing factors but the exact pathogenesis of CAD is not fully understood yet. However, increasing evidences suggest that inflammation plays an important role in the pathogenesis of both the chronic and acute phases of CAD [35,36.37].

The common cause of CAD is the formation of plaque and the rupture of the unstable atherosclerotic plaque. Recent molecular studies have shown altered mRNA level of many genes in both atherosclerotic plaque and peripheral blood cells that may be associated with CAD [39].

CAD is a chronic inflammatory disease. Atherosclerosis, the pathological formation of atherosclerotic plaques in one or more of the coronary arteries, is the leading cause of CAD [39].

Mediators of inflammation such as TNF-α have been associated with an increased risk for cardiovascular events in several clinical studies [40]. TNF-α is a central pro-inflammatory cytokine involved in the propagation of atherosclerosis. TNF-α is secreted in the vascular wall by endothelial smooth muscle cells and (monocytes/ macrophages) and it is a powerful inducer of local inflammation [41]. It promotes the expression of leukocyte adhesion molecules [42], and increases the uptake of macrophages in atherosclerotic lesions [43], thus directly promoting atherosclerosis.

The aim of the present study is to investigate serum concentration of IL-8 and TNF-α in Egyptian patients with coronary artery disease. Moreover, we aimed to clarify the possible relationships of IL-8, TNF α , diabetes, as well as other cardiovascular risk factors in Egyptian cardiac patients with different degrees of coronary artery occlusion.

Our study found that, diabetic patients with coronary artery disease had significantly higher values of systolic BP, diastolic BP, BMI, Waist-hip ratio, total cholesterol, TG , LDL.c , fasting blood glucose, fasting insulin, HbA1c and HOMA-IR compared to non-diabetic cardiac patients. Also the main finding of our results is that, IL-8, TNF α levels were significantly high in diabetic cardiac patients compared to non-diabetic ones. Moreover, when we stratified cardiac patients according to coronary artery occlusion we found that patients with three vessel occlusion had higher levels of inflammatory markers; IL-8 and TNF α.

Similar results were confirmed by **El-Hussieny et al.**, they observed elevation in TNF-α level in DM, HT and multi-risk factors CAD patients. In addition, a marked elevation in TNF-α plasma levels were reported in mild, moderate, and severe smokers, while the elevation was more significant in severe smokers than other groups [44]. These results are in agreement with **Popa et al.**, that recorded elevated TNF-α in congestive heart failure that was associated with atherosclerotic risk factors, including smoking, hyperglycemia, HT, and high LDL cholesterol [45].

**Feingold et al**., explained the role of inflammation in the development of metabolic syndrome features, including dyslipidemia and altered glucose tolerance. These metabolic changes constitute the substrate for the subsequent development of atherosclerotic plaque [46]. Chronic inflammatory conditions have been shown to be associated with pro-atherogenic lipid pattern and altered glucose tolerance. TNF-α has been demonstrated to directly interfere with metabolic pathways of triacylglyceride and cholesterol [47].

Our study investigated the influence of the main independent variables against IL-8 levels and TNF α levels in cardiac patients. Both were independently correlated with LDL .c, fasting blood glucose, waist /hip ratio , fasting insulin, HbA1c and HOMA-IR.

These findings were in close agreement with those reported by **Feingold et al**., who assessed the modifications that occur in lipid metabolism, TNF–α may interfere with glucose metabolism pathways [48], where it is likely to increase hepatic glucose production and decrease glucose uptake and catabolism in the muscle. While, in adipocytes, TNF-α down regulates the expression of several proteins implicated in the insulin receptor pathway [49].

This finding was consistent with the study by **Feingold et al.**, who explained the pro-atherogenic role of TNF-α and their effects on lipid and glucose metabolism in terms of both quality and quantity. Therefore, the persistence of these modified lipids in the circulation will promote the development of atherosclerotic lesions [46]. These data strongly support the hypothesis that the inflammatory cytokines (such as TNF-α) are surrogate biomarker of grade inflammation burden present in patients with atherosclerotic CAD who suffered from DM or HT or both of them [50].

Considering the results of our study, in patients with coronary artery disease, TNF-α was positively correlated with systolic BP, diastolic BP, total cholesterol ,LDL .c ,fasting blood glucose, fasting insulin, HbA1c and HOMA-IR . With regard to IL-8, in patients with coronary artery disease, IL-8 was positively correlated with systolic BP, diastolic BP, total cholestero ,LDL .c ,fasting blood glucose, fasting insulin, HbA1c and HOMA-IR.

In agreement with **El-Hussieny et al**., according to the number of stenotic coronary vessels, they reported an elevation in plasma TNF-α in CAD patients, where TNF-α is more significantly elevated in multi-vessels group than others. These data are supported by statistical analysis that showed a direct correlation between CAD and TNF-α level, which indicates the pivotal role played by TNF-α in atherosclerosis.

In accordance with our data, **Gotsman et al.**, previously found that the elevated plasma TNF-α concentration has correlation with severity of atherosclerosis [51].

On the contrary, **Hastings et al**., detected that neither IL-1β nor IL-8 induced proliferation or migration. Neutralization of the IL-8 receptor, CXCR2, further induced VCAM-1 in the presence of IL-1β, and phospho-p38 was required for NF-κB activation and VCAM-1 expression. Additionally, IL-8 reduced p38 activation and NF-κB activity induced by IL-1β alone.[52]

**In conclusion**, diabetic patients with coronary artery disease had significantly higher values of clinical and biochemical parameters of metabolic syndrome. Also, IL-8 and TNF α levels were significantly high in diabetic cardiac patients compared to non-diabetic one. Moreover, when we stratified cardiac patients according to coronary artery occlusion, we found that patients with three vessel occlusion had higher levels of inflammatory markers; IL-8 and TNF α and for further evaluation, IL-8 levels and TNF α

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levels in cardiac patients were independently correlated with LDL .c, fasting blood glucose, waist /hip ratio, fasting insulin, HbA1c and HOMA-IR by linear regression analysis. IL-8 and TNF $\alpha$ levels of cardiac patients were associated with diabetes and may be used as surrogate markers of CAD in diabetes and as strong predictive factors for progression of atherosclerosis in the coronary arteries.

## References


1. King H, Aubert RE, Herman WH. Global burden of diabetes,1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-1431.
2. Boyle JP, Honeycutt AA, Narayan KM, et al. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the U.S. Diabetes Care 2001;24:1936-1940.
3. Wingard DL, Barrer-Connor E. Heart disease and diabetes. In: National Diabetes Data Group, 2nd ed. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 1995:429-448.
4. Grundy SM, Howard B, Smith S Jr, Eckel R, Redberg R, Bonow RO. Prevention Conference VI: Diabetes and Cardiovascular Disease: executive summary: conference proceeding for healthcare professionals from a special writing group of the American Heart Association. Circulation 2002;105:2231-2239.
5. Butler WJ, Ostrander LD Jr, Carman WJ, Lamphiear DE. Mortality from coronary heart disease in the Tecumseh Study: long-term effect of diabetes mellitus, glucose tolerance and other risk factors. Am J Epidemiol 1985;121:541-547.
6. Klein R. Hyperglycemia and microvascular and macrovascular disease in diabetes. Diabetes Care 1995;18:258-268.
7. Kleinman JC, Donahue RP, Harris MI, Finucane FF, Madans JH, Brock DB. Mortality among diabetics in a national sample. Am J Epidemiol 1988;128:389-401.
8. Aronson D, Rayfield EJ, Chesebro JH. Mechanisms determining course and outcome of diabetic patients who have had acute myocardial infarction. Ann Intern Med 1997;126:296-306.
9. Gu K, Cowie CC, Harris MI. Diabetes and decline in heart disease mortality in US adults. JAMA 1999;281:1291-1297.
10. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002;105(9):1135–43.
11. Ghayour-Mobarhan M, Saber H, Ferns GA. The potential role of heat shock protein 27 in cardiovascular disease. Clin Chim Acta. 2012;413(1-2):15–24. doi: 10.1016/j.cca.2011.04.005.
2. Zernecke A, Shagdarsuren E, Weber C. Chemokines in atherosclerosis: An update. Arterioscler Thromb Vasc Biol. 2008;28(11):1897–908. doi: 10.1161/ATVBAHA.107.161174.
13. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105(9): 1135-43.
14. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol 2011; 12(3): 204-12.
15. Martins TB, Anderson JL, Muhlestein JB, Horne BD, Carlquist JF, Roberts WL, et al. Risk factor analysis of plasma cytokines in patients with coronary artery disease by a multiplexed fluorescent immunoassay. Am J Clin Pathol. 2006;125(6):906–13.
16 Hastings NE, Simmers MB, McDonald OG, Wamhoff BR, Blackman BR. Atherosclerosis-prone hemodynamics differentially regulates endothelial and smooth muscle cell phenotypes and promotes pro-inflammatory priming. Am J Physiol Cell Physiol. 2007;293:C1824–C1833.
17 Boisvert WA, Curtiss LK, Terkeltaub RA. Interleukin-8 and its receptor CXCR2 in atherosclerosis. Immunol Res 2000;21:129–37
18 -Fujishima S, Sasaki J, Shinozawa Y, Takuma K, Hori S, Aikawa N. Interleukin 8 in ARDS. Lancet 1993;342:237–8.
19 -Wang N, Tabas I, Winchester R, Ravalli S, Rabbani LE, Tall A. Interleukin 8 is induced by cholesterol loading of macrophages and expressed by macrophage foam cells in human atheroma. J Biol Chem 1996;271:8837–42.
20- Yue TL, Wang X, Sung CP, Olson B, McKenna PJ, Gu JL, et al. Interleukin-8. A mitogen and chemoattractant for vascular smooth muscle cells. Circ Res 1994;75:1–7.
21- Yue TL, Mckenna PJ, Gu JL, Feuerstein GZ. Interleukin-8 is chemotactic for vascular smooth muscle cells. Eur J Pharmacol 1993;240:81–4.
22 - Dominguez-Rodriguez A, Abreu-Gonzalez P, Garcia-Gonzalez M, Ferrer J. Prognostic value of interleukin-8 as a predictor of heart failure in patients with myocardial infarction and percutaneous intervention. Int J Cardiol 2006;111:158–60.
23 - Inoue T, Komoda H, Nonaka M, Kameda M, Uchida T, Node K. Interleukin-8 as an independent predictor of long-term clinical outcome in patients with coronary artery disease. Int J Cardiol 2008;124:319.
24- Kanda T, Hirao Y, Oshima S, Yuasa K, Taniguchi K, Nagai R, et al. Interleukin-8 as a sensitive marker of unstable coronary artery disease. Am J Cardiol 1996;77:304–7.
25 -American Diabetes Association. Consensus development conference on the diagnosis of coronary heart disease in people with diabetes. Diabetes Care 1998;21:1551-1559.
**26 -**Bruunsgaard H, Skinhoj P, Pedersen AN, Schroll M, Pedersen BK. Ageing, tumor necrosis factor alpha (TNF-alpha) and atherosclerosis. Clin Exp Immunol 2000; 121(2): 255-60.
27- Wang Q. Molecular genetics of coronary artery disease. Curr Opin Cardiol. 2005; 20(3): 182-188.
28- World Health Organization (WHO): The annual health report of the year 2009. Cairo, WHO, 2009.
29. Gunduz H, Demirtas S, Vatan MB, et al. Two cases of multi-vessel coronary artery ectasias resulting in acute inferior myocardial infarction. Korean Circ J. 2012;42:434–436
30. Raja SG. Myocardial revascularization for the elderly: current options, role of off-pump coronary artery bypass grafting and outcomes. Curr Cardiol Rev. 2012;8:26–36.
31. Lam CS, Ho KT. Coronary artery ectasia: a ten-year experience in a tertiary hospital in Singapore. Ann Acad Med Singapore. 2004;33:419–422.
32. Zografos TA, Korovesis S, Giazitzoglou E, et al. Clinical and angiographic characteristics of patients with coronary artery ectasia. Int J Cardiol. 2013;167:1536–1541.
33. Markis JE, Joffe CD, Cohn PF, et al. Clinical significance of coronary arterial ectasia. Am J Cardiol. 1976;37:217–222.
34.- Wang Q. Molecular genetics of coronary artery disease. Curr. Opin. Cardiol. 2005;20:182–188. doi: 10.1097/01.hco.0000160373.77190.
35-. Ross R. Atherosclerosis—An inflammatory disease. N. Engl. J. Med. 1999;340:115–126. doi: 10.1056/NEJM199901143400207.
36. Libby P. Inflammation in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 2012;32:2045–2051. doc
37-. Blanchet X., Cesarek K., Brandt J., Herwald H., Teupser D., Kuchenhoff H., Karshovska E., Mause S.F., Siess W., Wasmuth H., et al. Inflammatory role and prognostic value of platelet chemokines in acute coronary syndrome. Thromb. Haemost. 2014;112:1277–1287. doi: 10.1160/TH14-02-0139.
38-. Dabek J., Kulach A., Smolka G., Wilczok T., Scieszka J., Gasior Z. Expression of genes encoding kinin receptors in peripheral blood mononuclear cells from patients with acute coronary syndromes. Intern. Med. J. 2008;38:892–896. doi: 10.1111/j.1445-5994.2008.01718.x.
39. Fuster V., Fayad Z.A., Moreno P.R., Poon M., Corti R., Badimon J.J. Atherothrombosis and high-risk plaque: Part II: Approaches by noninvasive computed tomographic/magnetic resonance imaging. J. Am. Coll. Cardiol. 2005;46:1209–1218. doi: 10.1016/j.jacc.2005.03.075.
40- Ridker PM, Rifai N, Pfeffer M, Sacks F, Lepage S, Braunwald E. Elevation of tumor necrosis factor- alpha and increased risk of recurrent coronary events after myocardial infarction. Circulation. 2000; 101(18): 2149- 2153.
41- Libby P. Inflammation in atherosclerosis. Nature. 2002; 420(6917): 868-874.
42- Kim CJ, Khoo JC, Gillotte-Taylor K, Li A, Palinski W, Glass CK, et al. Polymerase chain reaction-based method for quantifying recruitment of monocytes to mouse atherosclerotic lesions in vivo: enhancement by tumor necrosis factor-alpha and interleukin- 1 beta. Arterioscler Thromb Vasc Biol. 2000; 20(8): 1976-82.
43- Skoog T, Dichtl W, Boquist S, Skoglund-Andersson C, Karpe F, Tang R et al. Plasma tumor necrosis factor- alpha and early carotid atherosclerosis in healthy middle-aged men. Eur Heart J. 2002; 23(5):376-383.
44- El-Hussieny EA, Gamal Eldeen AM, Talaat RM, Tamara AF, Salama MS, et al.: CD36 in Atherosclerotic Coronary Artery Disease: Correlation with Angiogenesis and Inflammation. SOJ Immunol. (2015) ; 3(2): 1-10. DOI:

Volume : 6 | Issue : 5 | May 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

45- Popa C, Netea MG, van Riel PL, van der Meer J W, Stalenhoef AF. The role of TNF-> in chronic inflammatory conditions, intermediary metabolism, and cardiovascular risk. J Lipid Res. 2007; 48(4): 751- 762.
46- Feingold KR, Pollock AS, Moser AH, Shigenaga JK, Grunfeld C. Discordant regulation of proteins of cholesterol metabolism during the acute phase response. J Lipid Res. 1995; 36(7): 1474-1482.
47- Memon RA, Grunfeld C, Moser AH, Feingold KR. Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. Endocrinology. 1993; 132(5): 2246-2253.
48- Feingold KR, Hardardottir I, Grunfeld C. Beneficial effects of cytokine induced hyperlipidemia. Z Ernahrungswiss. 1998; 37: 66-74.
49- Stephens JM, Pekala PH. Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. J Biol Chem. 1991; 266(32): 21839-21845.
50- Elneihoum AM, Falke P, Hedblad B, Lindgarde F, Ohlsson K. Leukocyte activation in atherosclerosis: correlation with risk factors. Atherosclerosis. 1997; 131(1): 79-84.
51- Gotsman I, Stabholz A, Planer D, Pugatsch T, Lapidus L, Novikov Y, et al. Serum Cytokine Tumor Necrosis Factor-Alpha and Interleukin 6 Associated with the severity of Coronary Artery Disease: Indicators of an Active Inflammatory Burden? Isr Med Assoc J. 2008; 10(7): 494- 498.
52- Nicole E. Hastings, Ryan E. Feaver, Monica Y. Lee, Brian R. Wamhoff, and Brett R. Blackman: Human IL-8 Regulates Smooth Muscle Cell VCAM-1 Expression in Response to Endothelial Cells Exposed to Atheroprone Flow. Arterioscler Thromb Vasc Biol. 2009 May; 29(5): 725–731