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Quantifying Serum TNF-Alpha Cut-Off Point for Predicting Coronary Stenosis Severity in a Population from Northern Iran

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Abstract: *Background:* Tumor necrosis factor-alpha has been linked to the severity of coronary artery disease. *Hypothesis*: To determine a cut-off point for TNF-alpha levels in predicting coronary artery disease (CAD) severity in a sample of patients from Northern Iran. *Methods*: 91 consecutive patients who were undergoing elective coronary angiography for suspected CAD at the Heshmat Heart Hospital in Northern Iran enrolled in this correlative cross-sectional study. The levels of TNF-alpha were determined using commercial ELISA kits. CAD severity was assessed by the Gensini and SYNTAX (SX) angiographic grading systems. Results revealed that in 86.8% and 71.4% of the patients had CAD based on the Gensini and SX scores, respectively. The mean level of TNF-alpha in CAD group was significantly higher than non-CAD group. Pearson's correlation coefficient test showed a positive linear correlation between the levels of TNF-alpha and Gensini ($r = 0.531$, $p < 0.001$), as well as SX scores ($r = 0.543$, $p < 0.001$). Multivariate regression model adjusting for age and sex showed that TNF-alpha was a strong predictor of CAD severity. According to the ROC curve analysis, TNF-alpha levels significantly discriminated between CAD and non-CAD condition diagnosed by Gensini and SX scores ($c = 0.752$ and.80, respectively) with the optimal cutoff point at 2.3 pg/ml, sensitivity ranging from 84.6% to 7.3% and the respected specificity of 50.0%. In Conclusion: Measuring serum TNF-alpha level is a highly sensitive and moderately specific test for predicting CAD severity at the estimated 2.3 pg/ml cutoff point in this specific sample.

Key words: Atherosclerosis · Coronary Disease · Coronary Angiography · Inflammation · Tumor Necrosis Factor

INTRODUCTION

Impairment of different components of immune system contributes to the atherosclerotic process from its early stages to plaque rupture. Recent studies have been focused on the role of some pro-inflammatory biomarkers involved in the pathological pathways of cardiac ischemia [1-3]. In this context, many of the immune cells exhibit signs of activation and produce inflammatory cytokines responsible for atheroma growth and expansion, leading to a matured atherosclerotic plaque [4-6].

Tumor necrosis factor-alpha (TNF-alpha) is a pluripotent cytokine produced by several types of cells, especially macrophages and is involved in systemic inflammatory conditions. It has been shown that patients with a variety of neoplastic, infectious and collagen vascular disorders exhibit elevated circulating levels of this non-glycosylated protein [7-11]. However, the potential role of TNF-alpha in cardiovascular disorders is already challenging. Elevation of TNF-alpha circulating levels in end stage heart failure has been reported [13]. Also, direct relationship between TNF alpha levels and

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functional heart failure classification has been demonstrated [14]. It was also suggested that TNF-alpha has negative inotropic effect mediated by inducing nitric oxide synthesis [15]. Moreover, provokes a hypertrophic growth response in cardiac myocytes, which may be an adaptive reaction to hemodynamic or environmental stress [16].

According to controversial reports on the association of serum TNF-alpha levels with extension and severity of CAD, we investigated whether a possible association existed between TNF-alpha levels and CAD severity assessed by Gensini and SYNTAX (SX) scores in a sample of Iranian patients with CAD.

MATERIALS AND METHODS

91 consecutive patients (41 male; mean age: 58.28 ± 9.34) who were undergoing elective coronary angiography for suspected CAD at Heshmat Hospital in Rasht enrolled in this study. Patients with myocardial infarction or any abnormal findings in ECG within recent 6 months, history of cerebrovascular or peripheral vascular diseases, alcohol consumption habit, malignancies, pregnancy, chronic inflammatory illnesses, or any cognitive disorders were not included. Upon entry, all the participants signed an informed consent. This study was reviewed and approved by the ethics committee of the Guilan University of Medical Sciences and adheres to the Declaration of Helsinki guidelines. Patients underwent an initial clinical assessment including interview for general characteristics, medical history and coronary risk factors, at the time of admission. Current smoking history (those who regularly smoke a tobacco product, one or more times per day or have smoked in the 30 days prior to admission) [17], hypercholesterolemia (total cholesterol ≥5.0 mmol/l, HDL-cholesterol ≥1.0 mmol/l in men, or ≥1.1 mmol/l in women and triglycerides ≥2.0 mmol/l) [18], family history of CAD (first-degree relatives before the age of 55 in men and 65 years in women) [19], hypertension (systolic blood pressure ≥140 mmHg and/or diastolic ≥90 mmHg and/or on antihypertensive treatment) [20], diabetes mellitus (symptoms of diabetes plus at least one of the following: plasma glucose concentration ≥11.1 mmol/l, fasting plasma glucose ≥7.0 mmol/l and a 2-hour postprandial blood glucose ≥11.1 mmol/l) [21], were taken. Body mass index was calculated from measured weight and height. Systolic and diastolic blood pressures were measured by standard mercury sphygmomanometer on the right arm in seated subjects and after 5 minutes resting. To measure TNF-alpha, 5.0 ml of venous blood samples were collected from each patient in the fasting state in the morning from an antecubital vein. The levels of TNF-alpha (pg/ml) were determined using commercial ELISA kits (Monobind, USA). All measurements were performed at the central laboratory of Heshmat Heart Hospital.

All the participants underwent a standard Seldinger technique for coronary angiography and a single cardiologist blind to the research protocol, analyzed the coronary angiograms. Images of the coronary tree were obtained in routine standardized projections with the digital Integris 3000 System (Siemens, Germany). Every lesion in the left main (LM), left anterior descending (LAD), left circumflex (LCX) and right coronary (RC) arteries was recorded. Patients were further classified as having one, two, or three-vessel disease depending on the number of coronary arteries with ≥50% stenosis. The Gensini scoring system was utilized for determining the severity of coronary lesions. A Gensini score was assumed significant when the LMCA showed ≥30%, or remaining vessels showed ≥50% stenosis. Multiple lesions in one vessel with at least 20 mm distance of each other were considered as a 2-vessel disease. The Gensini score was calculated for each patient from the coronary arteriogram by assigning a severity score to the degree of coronary stenosis with respect to the luminal narrowing and its anatomical importance [22].

For assessing CAD severity by SX score, each coronary lesion producing >50% luminal obstruction with ≥1.5 mm in length was separately scored and summated to provide the overall SX score which was calculated using dedicated software that integrates (a) the number of lesions with their specific weighting factors based on the amount of myocardium distal to the lesion and (b) the morphologic features of each single lesion. Higher SX score is indicative of more complex disease [23].

The primary endpoint of the study was assessing the increase in TNF-alpha parallel to the increase in CAD severity. The secondary endpoint of the study was to determine the best cutoff point for TNF-alpha to discriminate CAD from non-CAD status based on the two above diagnostic scores. Results were presented as mean ± standard deviation (SD) for quantitative variables and were summarized by absolute frequencies and percentages for categorical variables. Categorical variables were compared using chi-square test or Fisher's exact tests. Multivariate linear regression analysis was used to compare between-group differences in TNF-alpha levels controlling for demographic parameters and major CAD risk factors. A receiver operating characteristic (ROC) curve was used to identify the best cutoff point for

TNF alpha to discriminate CAD from non-CAD situations with optimal sensitivity and specificity. For the statistical analysis, the statistical software SPSS version 19.0 for windows (SPSS Inc., Chicago, IL) was used. A P value of 0.05 was assumed to be significant.

RESULTS

All the participants completed the study. Demographic characteristics of patients are summarized in Table 1. The mean age of study population was 58.28 ± 9.34 years and 45.1% were male (Table 1). The most and the least common CAD risk factors were hypertension (52.7%) and family history of CAD (3.3%), respectively. Percent of CAD positive subjects was higher using SX compared to Gensini scoring system. Regarding CAD severity, 79.1% of the patients showed mild and the remaining 20.9% had either moderate or severe forms of the disease. The mean levels of TNF-alpha were significantly higher in men than women, with no difference in subgroups with and without each CAD risk factors. In another words, serum TNF-alpha levels were not influenced by CAD risk factors except for male gender (P= 0.009, data not shown).

Considering Gensini score, the mean level of TNF-alpha in CAD group was significantly higher than the non-CAD group (38.08 ± 63.48 pg/ml versus 4.70 ± 7.76 pg/ml, p = 0.005). Similar difference was found according to the SX score (45.57 ± 67.74 pg/ml versus 3.96 ± 5.52 pg/ml, p = 0.001). Moreover, using Kruskal-Wallis test, the mean levels of TNF-alpha in patients with mild, moderate, or severe CAD assessed by SX scoring was 26.18 ± 51.98 pg/ml, 41.32 ± 47.21 pg/ml and 97.17 ± 127.24 pg/ml respectively which showed significant difference across the groups (p = 0.014).

Pearson's correlation coefficient test found a positive correlation between the level of TNF-alpha and Gensini score (r = 0.531, p < 0.001). Similar linear correlation was found between the level of TNF-alpha and SX score (r = 0.543, p < 0.001).

Multivariable linear regression analysis (Table 2) showed that the serum TNF-alpha level was a strong determinant for the presence of CAD measured by the Gensini score and adjusted for other study variables as cofounders. Also, the similar multivariate model (Table 3) showed that the serum TNF-alpha level could effectively predict CAD assessed by SX score.

According to the ROC curve analysis, serum TNF-alpha level had high values for discriminating between CAD and non-CAD condition based on Gensini (c = 0.752, 95% CI = 0.620 – 0.884) and SYNTAX score (c = 0.805, 95% CI = 0.715 – 0.894) (Fig. 1). The calculated cutoff point for TNF-alpha to discrimine between the patients with and without CAD in our specific sample was 2.3 pg/ml, yielding to a sensitivity of 87.3% with the use of Gensini and 84.6% with that of SX scoring systems and a specificity of 50.0%.

Table 1: Baseline characteristic and clinical condition of patients

Variable	Mean±SD	Variable	N %
Age	58.21±9.34	Male	41(45.1)
BMI*	28.8±4.27	Hyperlipidemia	44(48.4)
TNF alpha(pg/ml)	33.68±60.23	Hypertension	48(52.7)
Gensini score	24.32±26.9	Diabetes	30(33)
SX † score	12.39±14.21	Smoking	10(11)
		Family history	3(3.3)
		CAD+ Gensini	79(86.8)
		CAD+ SX	63(71.4)

* Body Mass Index

† SYNTAX

Table 2: Multi-variable regression model indicating the role of TNF- alpha for predicting CAD based on Gensini score

Variable	Standardized Beta	t score	P-value
TNF-alpha	0.477	4.860	<0.001
Male gender	0.174	1.441	0.153
Age	0.211	2.258	0.027
Body mass index	0.058	0.591	0.556
Hyperlipidemia	0.018	0.180	0.857
Hypertension	0.034	0.353	0.725
Diabetes mellitus	0.152	1.374	0.143
Cigarette smoking	0.079	0.785	0.435
Family history of CAD	0.016	0.169	0.867

Adjusted R square = 0.359

Table 3: Multivariate regression model indicating the role of TNF-alpha for predicting CAD based on SYNTAX score

Variable	Standardized Beta	t score	P-value
TNF-alpha	0.404	3.904	<0.001
Male gender	0.113	0.888	0.377
Age	0.188	1.907	0.060
Body mass index	-0.015	-0.144	0.886
Hyperlipidemia	-0.003	-0.030	0.976
Hypertension	0.018	0.178	0.859
Diabetes mellitus	0.209	1.796	0.076
Cigarette smoking	0.133	1.254	0.214
Family history of CAD	-0.051	-0.520	0.605

Adjusted R square = 0.287

DISCUSSION

Our study demonstrated an important role of serum TNF-alpha level to predict CAD and its severity. We found a positive correlation between circulating TNF-alpha levels and Gensini as well as SX scores in

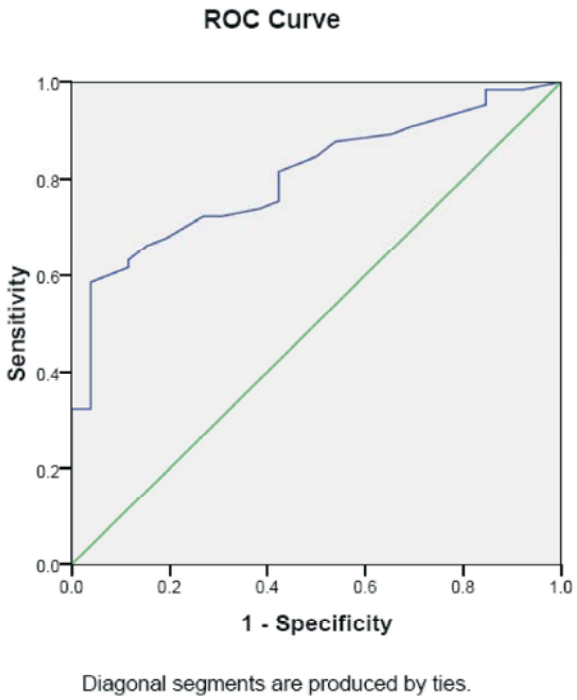

Fig. 1: ROC curves showing 86.7% sensitivity and 50% specificity for TNF alpha at 2.3 pg/ml cutoff point

assessing CAD extension. This investigation also showed high diagnostic value of TNF-alpha with an optimal cutoff point of 2.3 pg/ml and a corresponding sensitivity ranged from 84.6% to 87.3% and an estimated specificity of 50.0%. On the other hand, measuring this inflammatory biomarker can be considered as a highly sensitive and moderately specific test for predicting CAD severity. Similarly, Gotsman et al. showed a significant correlation between TNF-alpha and the severity of CAD assessed by the number of obstructed coronary vessels and the Gensini scoring system [24]. In another study by Lu *et al*., levels of this biomarker were correlated significantly with the number of diseased coronary vessels [25]. In previous studies, elevated serum TNF-alpha has been observed in those with cardiomyopathy, myocardial infarction and chronic heart failure and therefore implicating the role of TNF-alpha in disease pathogenesis [26]. Moreover, Grigoriadi and his co-workers in a recent study showed that TNF-alpha among other inflammatory biomarkers has a predictive value in the development of paroxysmal atrial fibrillation [27[. Gari MA and Akbarpour M in two separate studies showed decreased serum levels of TNF-alpha, IL-6, C-reactive protein and Leptin following interventional aerobic exercise training in high risk patients which confirms the pathogenic role of these molecules in atherosclerotic coronary disease [28, 29]. TNF-alpha has been detected in about 88% of atheromatous plaques, however was absent from normal tissues [30].

Also, TNF-alpha expression could increase with the extension of the lesion, suggesting it may play a role in disease evolution [23]. Arbustini *et al*. showed that TNF-alpha was not existed in lipid-rich plaques, with or without thrombosis and it was absent in normal control coronary arteries either [31].

The net effect of TNF-alpha on cardiac function depends on the amount and duration of TNF-alpha expression. It has been suggested that short-term expression of this biomarker might be an adaptive response to stress, while long term expression may be maladaptive by producing cardiac decompensation [32]. Excessive levels of TNF-alpha can produce left ventricular dysfunction [33] and can result in cardiomyocyte loss through necrosis or apoptosis. TNF-alpha can also induce apoptosis directly via its receptor, or indirectly through stimulation of nitric oxide production [34]. Moreover, it seems that TNF-alpha can depress myocardial function through two major pathways including the nitric oxide-dependent pathway [35, 36] and the sphingomyelinase pathway [37].

We used multivariable regression models to overcome the possible effects of other CAD risk factor confounders on our primary outcome. It has been well known that TNF-alpha can impact lipid metabolism and hyper-triglyceridaemia by decreasing lipoprotein lipase activity in cultured adipocytes that results in the increased levels of triglycerides [38, 39]. In addition, TNF-alpha probably plays a pivotal role in obesity-related insulin resistance [40]. Thus, considering these risk profile to evaluate relation between TNF-alpha and CAD severity seems to be necessary.

CONCLUSION

Serum TNF-alpha predicted the CAD severity at the optimal cutoff point of 2.3 pg/ml, in this specific sample with 84.6% to 87.3% sensitivity and 50.0%.specificity properties.

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