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ASSESSMENT OF INTERLEUKIN 1β AND TUMOR NECROSIS FACTOR-α FOR EARLY DETECTION OF CARDIOVASCULAR DISEASES IN IRAQI DYSLIPIDEMIC PATIENTS BY ROC CURVE

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ABSTRACT
A study was conducted to investigate the differences in lipid profile for 28 dyslipidemic patients and 16 apparently healthy (control), in addition to evaluate the validity of interleukin 1β (1L-1β) and tumor necrosis factor-α(TNF-α) for early detection of cardiovascular diseases (CVD) in Iraqi dyslipidemic patients by using receiver operation characteristic curve (ROC curve). Results revealed that the patients' elevated levels of total cholesterol (TC) mean ± SD (226.64 ± 43.60 mg/dl) as compared with control (146.5±31.79 mg/dl). The patients median values of triglyceride (TG) (157.50 mg/dl), low density lipoprotein (LDL, 133.00 mg/dl) and very low density lipoprotein (VLDL, median: 32 mg/dl) were significantly (P=0.0001) higher than corresponding values 92.5 mg/dl, 78mg/dl and 18.50mg/dl in control respectively. On the other hand, dyslipidemic patients have significant (P < 0.0001) lower levels of HDL (median: 40.50 mg/dl) as compared with control (median: 51.50 mg/dl).Results also show that the levels of 1L-1β (median: 13.21 pg/ml) and TNF-α (mean ± SD: 43.56±9.59 pg/ml) in patients are released with significant (P <0.0001) high concentration for dyslipidemic as compared with corresponding levels in control (median: 5.50 pg/ml and mean±SD:24.70± 9.60pg/ml respectively).The sensitivity, specificity and area under curve (UAC)of 1L-1β were 0.89, 0.75, and 0.84. Whereas the corresponding values of TNF-α were 0.89, 0.81, and 0.92. These results confirmed their validity for detection of CVD. ROC curve was identified the cutoff point of ≥9.17 and ≥31.40 for 1L-1β and TNF-α respectively.

KEYWORDS: interleukin-1β, tumor necrosis factor-alpha, cardiovascular disease, dyslipidemia, atherosclerosis.

INTRODUCTION

Dyslipidemia is elevation of blood cholesterol, triglycerides or both, or a low high-density lipoprotein level that contribute to the development of atherosclerosis, dislipidemia consider a disorder of lipoprotein metabolism, including overproduction or deficiency, cause no symptoms but can lead to symptomatic vascular disease, like myocardinal infarction (heart attack) stroke, and peripheral arterial disease[1].Triglycerides is a type of fat, supply the body with energy but high blood level can raise the risk of heart diseases or cause metabolic syndrome, also associated with other sclerosis even in the absence of hyper cholesterolemia[2]. Low-density lipoprotein (LDL) called bad kind of cholesterol. LDL carries the majority of cholesterol in the blood stream, and it is the main lipid that accumulates in arterial plaques [3].
High-density lipoprotein (HDL), conversely, carry LDL-cholesterol out of the blood stream and in to the liver, where it is metabolized and cannot block blood vessels, in a process known as reverse cholesterol transport[4].An artery wall thickens as a result of the accumulation of fatty materials such as cholesterol and triglycerides, it reduces the elasticity of the artery walls and therefore allows less blood to travel through this chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophages and white blood cells and promoted by LDL (that carry both cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional HDL. So this leads hardening of the arteries, it is caused by the formation of multiple plaques within the artery [5]. Serum lipid profile for both dyslipidemic patients and healthy controls were estimated. Increased levels of proinflammatory cytokines like interleukin-1beta (1L-1β) and tumor necrosis factor alpha (TNF-α) found within atheromatous plaque. The release of 1L-1β from monocytes initiates the systemic inflammatory response. An intense 1L-1β dependent response occurs during acute myocardial infarction and promotes heart failure, a chronic 1L-1β dependent process alters cardiovascular function leading to impaired performance, poor quality of life and increased morbidity and mortality[6]. TNF-α is a proinflammatory cytokine with potent stimulatory effects in immune and vascular response[7]. It is released from activated monocyte, mast cell, endothelial cells and mainly from macrophages[8]. The main function of TNF-α is the control of acute phase reaction with the release of acute phase reactants from the liver and induction of cytokines[8]. TNF-α affects lipid metabolism, coagulation and endothelial function, the increasing release of TNF-α might be expected to associated with increased stroke risks[9], in addition to a variety of human diseases[10]. Atherosclerosis is associated with activation of the inflammatory process and with systemic increase of proinflammatory molecules like 1L-1β and TNF-α and thus it turns from a disease caused by a simple accumulation of lipid in to a complex disorder influenced by the inflammatory response of the arterial

wall. The effect of proinflammatory molecules is attributed to their ability to modulate a number of key events involved in the complex inflammatory process of atherogenesis such as the vessel wall inflammation, leukocyte chemotaxis, adhesion or plaque rupture [11]. A comparison study was established for serum 1L-1β and TNF-α of both dyslipidemic patients and healthy controls.Different static studies were used for the current data including Shapiro-Wilk test, t-test, and Wilcoxon test. The determination of cut off point by evaluation of both sensitivity and specificity that shows the validity of 1L-1β and TNF-α parameters to detect cardiovascular diseases.In addition area under the cure (AUC) was evaluated for both 1L-1β and TNF-α. Results also confirmed the validation of the two parameters for detection of cardiovascular diseases.

PATIENTS & METHODS

The study was conducted in the AL-Yarmok Teaching Hospital. 28 patients with dyslipidemia (16 females, 12 males) age range (43-64) years, and 16 apparently healthy controls (9 females, 7 males) age range (37-52). The estimation of lipid profile was carried for both groups. Enzymatic colorimetric method for determination of cholesterol, by addition of an enzyme mixture [cholesterol esterase (CHE), cholesterol oxidize (CHOD) and peroxides (POD)].The cholesterol in the sample originates a colored complex (Quinonimine) compared with standard by reading the absorbance (A) of both sample and standard at 505 nm. Also enzymatic colorimetric method for triglycerides, in which the sample triglycerides incubated with lipoprotein lipase (LPL). Liberate glycerol and free fatty acid, and by the action of glycerol kinas (GK) and glycerol 3-oxidase (GPO) glycerol liberate hydrogen peroxide (H_2O_2) that reacts with 4-aminophenazone give a red colored complex and the intensity of the color is proportional to the triglycerides concentration in the sample which also compare with standard. Manual precipitant for HDL (high density lipoprotein), by the addition of phosphotungstic acid in the presence of magnesium ion, the low density lipoproteins (LDL and VLDL) and chylomicron fraction are precipitated, After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant is determined. Total cholesterol is defined as the sum of HDL, LDL, and VLDL. Usually, only the total, HDL and triglycerides are measured. The VLDL is usually estimated as one-fifth of the triglycerides and the LDL is estimated using the Friedewald formula:

Estimated LDL=[total cholesterol]-[total HDL]-[estimated VLDL].

The values of LD; that obtained using this assay are reliable provided that no chylomicrons are present in the sample, the triglyceride concentration does not exceed 400mg/dl [12]. Elisa kit for determination of (1L-1β)[13], in this kit the micro titer. Plate has been pre-coated with an antibody specific to 1L-1β. Standards or samples are then added to the appropriate micro titer plate wells with a biotin-conjugated polyclonal antibody preparation specific for 1L-1β and Avidin conjugated to Horseradish peroxides (HRP) is added to each micro-plate well and incubated. Then a TMB (3, 3`, 5, 5`) tetra methyl benzidine substrate solution is added to each well. Only those wells that contain 1L-1β, biotin-conjugated antibody and enzyme conjugated Avidin will exhibit a change in color which measured spectra-photo metrically at 450nm. The concentration of 1L-1β in the sample is then determined by comparing of O.D. of the sample to the standard curve. Tumor necrosis factor (TNF-α)[14], enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. During the first incubation period TNF-α in samples are captured by the monoclonal antibody to human TNF-α coated on the wall of the microtiter wells. After washing away the unbound components from samples, a peroxidase-labeled second monoclonal antibody conjugated is added to each well and then incubated. After a second washing step, the bound enzymatic activity is detected by addition of tetrametlyl benzidine (TMB) chormogen-substrate. Finally, the reaction is teriminated with an acidic stop solution and the optical density is measured with a photometer at 450nm. The intensity of the color is directly proportional to the concentration of human TNF-α in sample. The result is expressed as pg/ml.

Statistical Analysis

Analysis of data was performed by SAS (9.1) program, for testing normality data were subjected to Shapiro-Wilk test. According to this test, only two parameters have normal distribution (TC and TNF-α). Hence, the significant differences between groups for those two parameters were conducted by using t-test. Whereas the other parameters have not normal distribution and the differences between parameters were performed by Wilcoxon-Mann-Whitney test, which is a non-parametric test analog to the independent samples t-test. Data were also subjected to Receiver Operation Characteristic curve (ROC curve) to evaluate the validation of IL-lβ and TNF-α to detect CVD in Iraqi dyslipidemic patients.

RESULTS

Normality of data was tested according to Shapiro-Wilk test. Results revealed that only total cholesterol (TC) and tumor necrosis factor-alpha (TNF-α) parameters have normal distribution. The difference between two parameters was tested by t-test, whereas the other parameters were tested by Wilcoxon test. The levels (TC, TG. LDL, VLDL, IL-lβ and TNF-α) were elevated significantly in patients as compared with control. On the other hand, the level of HDL was lowered significantly in patients as compared with the controls as shown in table 1 and 2, respectively.

TABLE 1: Shapiro-Wilk test for normality of all parameters

	W-Statistic	P
Total chol/Patient	0.94	0.18
Total chol/control	0.90	0.11
TG Patient	0.91	0.02
TG control	0.58	0.0001
HDL Patient	0.85	0.001
HDL control	0.88	0.04
LDL Patient	0.93	0.10
LDL control	0.88	0.04
vLDL Patient	0.92	0.03
vLDL control	0.65	0.0001
IL-lβ Patient	0.75	0.0001
IL-lβ control	0.87	0.03
TNF-α Patient	0.98	0.96
TNF-α control	0.95	0.50

Result is significant if $P \leq 0.05$.

TABLE 2: Medians, means and standard deviation for all parameters (dyslipidemic patients and healthy control)

Parameter	STATUS	N	Median	Wilcoxon	Mean	SD	t-test
TC	Patient	28			226.64	43.60	0.0001
mg/dl	control	16			146.50	31.79	
TG	Patient	28	157.50	0.0001			
mg/dl	control	16	92.50				
HDL	Patient	28	40.50	0.0001			
mg/dl	control	16	51.50				
LDL	Patient	28	133.00	0.0001			
mg/dl	control	16	78.00				
VLDL mg/dl	Patient	28	32.00	0.0001			
	control	16	18.50				
1L-1β	Patient	28	13.21	0.0002			
pg/ml	control	16	5.50				
TNF-α	Patient	28			43.56	9.59	0.0001
pg/ml	control	16			24.70	9.60	

Results of ROC curve confirmed the validation of IL-lβ and TNF-α to detect CVD in Iraqi dyslipidemic patients. The value of cut off point of IL-lβ was ≥9.17 with sensitivity 0.89, specificity 0.75 and area under curve (AUC) 0.84. The corresponding values for TNF-α were ≥31.40, 0.89, 0.81 and 0.92 respectively (table 3 and 4). The estimated ROC curve of IL-lβ and TNF-α is illustrated in figure (1).

TABLE 3: Sensitivity, Specificity and cut off point for 1L-1β and TNF-α

	Estimation	Cut off point
Sensitivity/IL-1β	0.89	
Specificity/IL-lβ	0.75	≥9.17 pg/ml
Sensitivity /TNF-α	0.89	
Specificity/TNF-α	0.81	≥31.40 pg/ml

TABLE 4: Area Under Curve for 1L-1β and TNF-α

Test Result Variable(s)	AUC	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Lower Bound
1L-1β	0.847	0.069	0.000	0.713	.981
TNF-α	0.920	0.040	0.000	0.842	.998

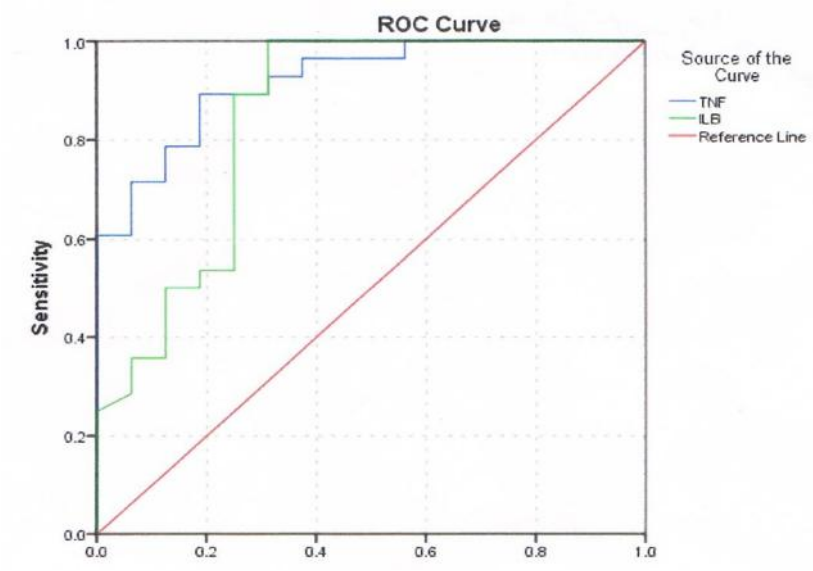

FIGURE 1: ROC curve for 1L-1β and TNF-α

DISCUSSION

Dyslipidemia considered the major cause of the accumulation of atherogenic lipoprotein in blood useless and block circulation. Atherosclerosis initiated by inflammatory processes in the endothelial cells of the vessel wall in response to retained LDL molecules[15], LDL particles and their content are susceptible to oxidation by free radicals[16]. The damage cause by the oxidized LDL molecules triggers a cascade of immune responses which over time can produce an atheroma, this leading to narrowing of the artery, reducing blood flow and increase blood pressure[16], HDL plays an important role, in the reverse cholesterol transport as well as having anti-inflammatory and ant oxidative effects. Dysfunction of HDL is an independent pro-atheroqenic factor[17]. Dyslipidemia itself usually causes no symptoms but can lead to symptomatic vascular disease including coronary artery disease, stroke and peripheral arterial disease, as a result of atherosclerosis. Circulating cytokines interact with specific receptors on various cell types and activate signaling path ways leading to an inflammatory response involving cell adhesion, permeability and apoptosis [18]. Atherosclerosis is a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophages that releases different pro-inflammatory cytokines like 1L-1β and TNF-α. Inflammation has important roles in the development and rupture of atherosclerotic lesions leading to CVD events [19]. 1L-1β is involved in pathogenesis of different cardiovascular diseases due to its proinflammatory potential, that effect on cardiac myocytes and later on the progression of atherosclerosis [20].

The TNF-α also as a proinflammatory cytokine with potent stimulatory effects in immune and vascular response[21], and high circulating levels of TNF-α might be expected to be associated with increased stroke risks[22], which is caused by blockage of majority arteries. Prolonged ischemia or oxygen shortage in consequence of an insufficient blood supply may result in damage and necrosis of the affected tissue[23], such an event is likely to be followed by sterile inflammation triggered by a variety of molecules released from dying cells. Cytokines are master regulators of the innate and adaptive immune response also are known to regulate and, essentially, coordinate many stages of atherosclerosis [24].

The current study shows increased circulating levels of both 1L-1β and TNF-α for dyslipidemic patients in comparison with that of controls levels for both cytokines. Results confirmed its validity for 1L-1β and TNF-α to detect the CVD in blood concentration (≥9.17 pg/ml for 1L-1β and ≥31.4pg/ml for TNF-α). Both of 1L-1β and TNF-α are good biomarkers and the determination of blood concentration for them could be valuable tool for dyslipidemic patients as early diagnosis of CVD. Since atherosclerosis is often asymptomatic, narrowing of an artery. Signs and symptoms usually come out when the severe blockage impede-blood flow to different organs[25], and these symptoms varies depending on which artery or organ is affected[26], causing different cardiovascular disorders like heart attack and stroke.

CONCLUSION

Atherosclerosis is greatly influenced by inflammatory mediators at all phases in the development of atherosclerotic vascular disease and the potential of its inhibition as promising therapeutic strategy for the treatment of atherosclerotic vascular disease[27]. By the use of human monoclonal antibody that binds human cytokine thus blocks the interaction of this cytokine with its receptors [28].

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