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RESEARCH ARTICLE

THE CARDIO-PROTECTIVE "PARAOXONASE-1" AS A VALUABLE PROMISING TOOL IN DIAGNOSIS AND/OR ASSESSMENT OF CARDIOVASCULAR RISK IN TYPE 2 DIABETES.

Ahmed M. A. Akabawy[1], Heba I. Hamed[2], Wafaa S. Hegab[3] and Naglaa F. Mohamed[4].

1. Faculty of Pharmacy, Helwan University, Department of Biochemistry and Molecular Biology, Cairo, Egypt.
2. National Institute for diabetes and Endocrinology, Department of Biochemistry, Cairo, Egypt.
3. National Institute for diabetes and Endocrinology, Department of Internal medicine, Cairo, Egypt.
4. National Institute for diabetes and Endocrinology, Department of Clinical & chemical pathology, Cairo, Egypt.

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Abstract

Background: Type 2 Diabetes Mellitus "T2D" remains frequently undiagnosed for many years; as the hyperglycemia is often not severe enough to provoke noticeable symptoms. Thus, such patients are at increased risk of developing macrovascular and microvascular events. Diabetic cardiovascular "CVD" complications are one of the most leading causes of death worldwide. Prevention of long-term chronic complications has become one of the main goals of modern treatment in T2D. However, new tools are still needed for early detection or even prediction of CVD risk to facilitate decreasing morbidity and mortality rates associated with T2D.

Objectives: In this study, authors aimed to afford access to information that may allow early detection &/or prediction of macrovascular complications in Egyptian patients with T2D.

Subjects and Methods: The study included 200 clinically diagnosed T2D patients (subdivided according to presence of CVD complication). In addition, 60 healthy controls were selected with comparable socioeconomic, age, body mass index, and sex distribution. Inclusion and exclusion criteria were applied. Glycemic control indices, lipid profile, Paraoxonase-1, MCP-1, ICAM-1, VCAM 1, and Lp (a) were measured. Results were statistically analyzed.

Results: Serum levels of Paraoxonase-1 (↓), MCP-1 (↑), ICAM-1 (↑), VCAM 1 (↑), and Lp (a) (↑) were altered significantly in both diabetic cohorts compared to healthy controls. Also, Paraoxonase-1 (↓↓), MCP-1 (↑↑), ICAM-1 (↑↑), VCAM 1 (↑↑) levels showed significant alteration in T2D patients with CVD compared to those without complication. Additionally, with respect to prediction of CVD risk, results predict that Paraoxonase-1 has higher and better diagnostic accuracy index as indicated by ROC analysis.

Conclusion: Paraoxonase-1 is a sensitive biomarker in T2D with anti-atherogenic cardio-protective properties; its circulating levels may be a valuable future tool for early diagnosis and/or assessment of CVD risk associated with T2D.

Corresponding Author: Ahmed M. A. Akabawy
Address: Faculty of Pharmacy, Helwan University, Biochemistry and Molecular Biology Depart., Cairo, Egypt.

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Introduction:-

Diabetes mellitus is associated with increased risk of complications including micro- and macro-vascular complications which are the major cause of morbidity and mortality ***(Bo et al., 2006)***. The primary causal factor leading to the pathophysiologic alterations in the diabetic vasculature is chronic exposure to hyperglycemia ***(Savita et al., 2013)***. Cardiovascular disease (CVD) is one of the first leading causes of death worldwide, especially in patients with type 2 diabetes mellitus (T2D). Patients with T2D have a high mortality risk related to CVD ***(Ruderman et al., 1992)***. The risk of developing T2D is partly attributed to an increased prevalence of classic coronary heart disease (CHD) risk factors, hyperglycemia and a highly atherogenic lipid profile. Despite that, the CVD are multifactorial disorder where environmental, dietary habits and lifestyles play important roles in pathogenesis.

PONs, including PON1, PON2 and PON3, are a series of serum esterase enzymes synthesized in the liver.PON1 and PON3 are secreted from the liver into the blood circulation and is associated with HDL particles ***(van Himbergen et al., 2006)***. In ***2006, Rosenblat et al.*** described the role of PON1 hydrolytic activity to mediate inhibition of low-density lipoprotein (LDL) oxidation and stimulation of cholesterol efflux from macrophages. Authors also reported that apolipoprotein (Apo) A-I in HDL stimulates PON1 lactonase activity. PON1 is implicated in preventing atherogenesis and CHD ***(Marta et al., 2004, Goswami et al., 2009).*** PON1 activity was studied in many diseases; regarding the role of PON1 in renal diseases, a study showed that PON1 activity was decreased in hemodialysis patients, especially in elderly ones (***Hanaa et al., 2008)***. Activity of PON1 is also found to be altered also in patients with T1D or T2D ***(Mackness et al., 2000, Boemi et al., 2001).***

Prospective studies suggest that inflammation is involved in the pathogenesis of diabetes and atherosclerosis ***(Ross, 1999).*** Hence, measurement of inflammation sensitive markers may be useful for the assessment of CVD risk in diabetic patients as well as in the general population. Monocyte chemoattractant protein-1 (MCP-1), a member of the CC subgroup of the chemokine superfamily, is involved in the recruitment of monocytes into the arterial vessel wall ***(Gokulakrishnan et al., 2008).*** MCP-1 levels were found to be increased in aging ***(Inadera et al., 1999)***, hypertension ***(Parissis et al., 2000)***, hypercholesterolemia ***(Garlichs et al., 2001)***, and renal failure ***(Papayianni et al., 2002).*** Also, MCP-1 has a prognostic value in the acute and chronic phases in patients with acute coronary syndromes ***(de Lemos et al., 2003, de Lemos et al., 2007)***.

The leukocytes adhesion and their trans-endothelial migration play an important role in the initial phase of atherogenesis ***(Cybulsky et al., 1991)***. Processes are regulated by the various types of adhesion molecules, such as the intercellular adhesion molecule 1 (ICAM-1), the vascular cell adhesion molecule-1 (VCAM-1) and the platelet endothelial celladhesion molecule-1. Their expression takes place on the surface of endothelial cells, hematopoetic cells, and immunological cells ***(Gearing and Newman, 1993)***. ICAM-1 is a transmembrane glycoprotein receptor consisting of 505 amino acids, with molecular weight between 80 and 114 KDa depending on the degree of glycosylation ***(Newman et al., 1990)***. ICAM-1 belongs to the immunoglobulin superfamily. It acts as a ligand β 2-integrin present on leukocytes. It consists of five extracellular domains, a transmembrane domain and a short cytoplasmic domain ***(Staunton et al., 1988)***. Increased concentrations of ICAM-1 were reported in patients with CVD, tumors, autoimmune diseases, and other diseases with an inflammatory reaction ***(Witkowska and Borawska, 2004)***.

VCAM-1 is a glycosylated cell surface protein expressed on different cell types, including endothelial cells and fibroblasts. VCAM-1 is a member of the immunoglobulin (Ig) gene superfamily with seven cell surface Ig-like domains, a hydrophobic transmembrane domain and a short cytoplasmic domain of 19 amino acids ***(Osborn et al., 1989)***. Elevated expression of VCAM-1 on endothelial cells depends on the presence of cytokines and mediates leukocyte accumulation in inflamed tissues ***(Carlos and Harlan, 1994).*** The predominant receptor for VCAM-1 is the integrin α4β1, while α4β7 and plasmodium falciparum-infected erythrocytes also function as VCAM-1 receptors ***(Elices et al., 1990, Chan et al., 1992, Ockenhouse et al., 1992)***.

Lipoprotein (a) [Lp (a)] is a complex human plasma lipoprotein that consists of two molecules, an LDL and an apo B-100 particle, linked to the plasminogen-like apo (a) via a disulfide bridge ***(Ridker et al., 2012)***. The Lp (a) synthesis is mainly regulated by the apo (a) gene, which is located on chromosome 6q26-27. There is a wide heterogeneity of Lp (a) mainly due to kringle IV-type 2 repeats in the apo (a) gene, each of which consists of

different sized apo (a) proteins called isoforms. The size of these proteins is generally inversely correlated to the Lp (a) concentration in the plasma ***(van der Hoek et al., 1993)***. Lp (a) synthesis is separated from that of LDL and occurs mainly in the liver; Apo (a) is produced by liver cells and then assembled with LDL particles on the hepatocyte surface, perhaps on LDL receptors ***(Koschinsky and Marcovina, 1997)***. However, LDL receptors do not seem to be relevant in the Lp (a) catabolism. Some theories suggest that Lp (a) removal takes place through other mechanisms, such as urinary elimination ***(Albers et al., 2007)*** or via macrophage scavenger receptors ***(Argraves et al., 1997)***. **In this study, authors aimed to afford access to information that may allow early prediction of macrovascular complications in type 2 Egyptian diabetic patients.**

Subjects and Methods:-

Subjects:-

The study was performed on 200 outpatients with T2D and 60 healthy control subjects. All patients were selected from the outpatients' clinic of the NIDE, Cairo, Egypt. Diagnosis of diabetes was based on current criteria of the

American Diabetes Association, 2010.

Inclusion criteria:

Inclusion criteria of patients include clinical diagnosis with T2D, age between 35 and 60 years, A1C level > 7 % and diabetes duration ≥ 3 years. In respected patients, presence of CVD evidence should start after at least 3 years of T2D diagnosis. Presence of CVD complication in patients with T2D is defined as the clinical presentation with evidence for CHD, congestive heart failure, and/or electrocardiograph evidence of myocardial infarction or coronary angiography. All patients were taking the same type of hypoglycemic therapy. Demographic data was recorded for each subject using self-made questionnaire. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and healthy subjects that described the aim of the study and the procedures that would be required from them. Participants were further divided into three groups, *group 1* "*C gp*": healthy control group (n=60), *group 2* "*Do gp*": Patients with T2D without evidence of CVD complications (n=110), and *group 3* "*Dc gp*": Patients with T2D with CVD complication (n=90). The following variables were also recorded: age, sex, duration of disease, body Mass Index (BMI) and blood pressure. BMI was calculated as weight (kg) divided by height squared meter (Kg/m^2) according to ***Shiwaku et al., 2004***. Blood pressure measurements were performed by trained technicians or nurses with a mercury sphygmomanometer and the first and fifth Korotkoff sounds were recorded to represent the systolic and diastolic pressure; two measurements were obtained and averaged. Hypertension was considered if the systolic blood pressure was G140 mm Hg or diastolic blood pressure G90 mm Hg or use of medication for hypertension.

Methods:-

Blood samples were collected after 12 hr overnight fasting from all subjects into three types of vacutainer tubes. First tube is without additives; blood was centrifuged at 3000 rpm for 10 minutes. Serum was rapidly separated for determination of lipid profile, serum creatinine at once and the remaining serum aliquots was stored at -80°C until the measurements of serum Paraoxonase-1, MCP-1, ICAM-1, VCAM-1 and Lp (a); hemolyzed samples were excluded. Second part of the collected blood was taken on EDTA-containing tubes for immediate assaying of A1C level using a standard ion exchange HPLC method on G8 HPLC analyzer *(Tosoh, Tokyo, Japan)*. Third part of collected blood was taken onto fluoride-containing tubes for determination of fasting plasma glucose (FPG) level at once utilizing glucose oxidase method according to ***Barham and Trinder, 1972***. Serum total cholesterol (TC) was determined by the enzymatic method according to ***Allain et al., 1974***. Triacylglycerol (TAG) was assayed by peroxidase-coupled method according to ***Mc Gowan et al., 1983***. HDL-c was measured by enzymatic method according to ***Finley et al., 1978***. LDL-c was measured according to ***Friedewald et al., 1972***. Serum creatinine was measured by colorimetric method according to ***Vasiliades, 1976***. Sampling, reagent delivery, mixing, processing, calculating and printing were full automatically performed by BT3500 chemistry system *(Biotecnica, Instruments Inc, ITALY)*. Serum human Paraoxonase-1 and ICAM-1 concentrations were measured using commercially available ELISA Kits - Glory Science (Catalog No #:95462 and #:10163 respectively) *(Glory Science Co., USA)*. Serum human MCP-1 and VCAM-1 concentrations were measured using commercially available ELISA Kits (Catalog No DCP00 and DVC00 respectively) *(R&D systems, Minneapolis, MN, USA)*. Serum human Lp (a) concentration was measured using commercially available ELISA Kit - DRG (Catalog EIA-4406) *(DRG International Inc., USA)*.

Statistical analysis:

Data was expressed as the M ± SD. Statistics were calculated, and appropriate graphs and histograms were plotted when needed for the entire study cohort, using GraphPad Prism 5 (For Windows, © 1992-2007 Graphpad software Inc., V 5.01, USA) which was used to test the significance of differences between groups in the present study. To analyze more than two sets of data, ordinary one way analysis of variance (ANOVA) for parametric data was first tried, followed by Tukey-Kramer multiple comparison test. Furthermore, analysis was performed to examine the possibility for any correlation between different parameters. For clinical correlations, the correlation co-efficient was calculated using least square method.

Results:-

Clinical data and demographic measures for patients and controls are shown in table (1). Concerning gender, BMI and age, in-significant variations were verified when diabetic and healthy control cohorts were compared using Chi-squared and one-way ANOVA respectively ($p > 0.5$). Levels for both glycemic control indices (FPG & A1C) were significantly increased in a parallel manner (levels in C gp. < Do gp. < Dc gp.). Significant multiple comparisons were ensured when Tukey-Kramer post-Hoc test was applied ($P < 0.05$).

As seen in table (1) and fig. (1); serum levels of both Paraoxonase-1 and HDL-c were significantly decreased in both diabetic cohorts compared to healthy controls ($P < 0.001$). Only for Paraoxonase-1, levels also showed a significant decrease in the Dc gp. if compared with Do gp. ($P < 0.01$). In contrary to the pattern showed by Paraoxonase-1 serum concentrations in the studied groups, levels of MCP-1, ICAM-1, VCAM-1, TC, TAG and LDL-C showed a significant gradual increase from C gp. passing through Do gp. reaching their highest levels in the Dc gp. All multiple comparisons were significantly verified using post-hoc tests ($P < 0.01$). Regarding Lp (a), although the same behavior was seen, the significance was noted only if either diabetic group was compared with healthy controls ($P < 0.001$) but no significance was shown between Do and Dc groups.

Pearson correlations are listed in table (2). Excluding Paraoxonase-1 and HDL-c, serum levels of MCP-1, ICAM-1, VCAM-1 and Lp (a) were positively correlated with either any investigated parameters ($P < 0.01$). On the contrary, Paraoxonase-1 showed a negative correlation when tested against all investigated parameters ($P < 0.001$) except for HDL-c (Positive correlation at $P < 0.001$). Glycemic indices and lipid profile data were also significantly correlated as shown in table 2.

Receiver operating characteristic curve "ROC": *Qualitatively,* the closer the curve, to the top left-hand corner, the higher the overall accuracy of the test is. *Quantitatively,* the area under the curve "AUC" is an overall measurement of the accuracy. Paraoxonase-1 was very close to the top left-hand corner showing AUC ± SEM; 0.94 ± 0.03. HDL/LDL ratio has lower AUC ± SEM value; 0.78 ± 0.06. Data are represented in fig. (2).

Table 1:-Demographic characteristics and mean serum levels of investigated parameters among studied groups

Parameters	*C gp. (№ 60)*	*Do gp. (№ 110)*	*Dc gp. (№ 90)*
Age (years)	43.53±6.63	43.0±6.67	44.53±4.54
BMI (Kg/m^2)	35.4±4.11	34.75±3.12	35.4±4.46
Disease Duration (years)	----------	7.9±3.5	8.63±4.43
Gender (male/female)	18 / 42	26 / 84	27 / 63
FPG (mg/dl)	90.23±5.92	203.4±63.06 ***	234.6±43.77 ***, #
A1C (%)	5.26±0.25	8.37±1.53 ***	9.27±1.56 ***, #
TC (mg/dl)	184.3±21.73	207.43±27.97 **	234.5±30.47 ***, ##
TAG (mg/dl)	92.0±22.45	171.67±22.63 ***	232.33±45.95 ***, ###
HDL-c (mg/dl)	54.7±4.4	38.73±6.5 ***	36.07±5.03 ***
LDL-c (mg/dl)	109.07±13.68	136.93±21.55 ***	167.77±23.62 ***, ###
HDL/LDL ratio	0.51±0.09	0.31±0.08***	0.23±0.07 ***, ##
Paraoxonase-1(nmol/l)	41.91±14.09	29.19±3.5 ***	21.15±4.48 ***,##
MCP-1 (pg/ml)	150.62±27.95	275.78±105.84 ***	356.87±92.0 ***.##
ICAM-1 (ng/ml)	149.0±18.57	209.9±43.72 ***	285.75±67.74 ***,###
VCAM -1 (ng/ml)	239.67±38.41	390.92±72.2 ***	493.26±159.36 ***,##
Lp (a) (mg/dl)	16227.87±2512.66	22754.9±1882.41 ***	23513.6±3036.56 ***

- Data are expressed as mean ± SD and approximated to the second decimal.
- C gp., Do gp., Dc gp.: Healthy control group, Type 2 diabetic patients without complications, Type 2 diabetic patients with CVD.
- *: p < 0.05, **: p < 0.01, ***: p < 0.001 when compared to *C gp.* using one-way ANOVA followed by Tukey Kramer multiple comparison test.
- #: p < 0.05, ##: p < 0.01, ###: p < 0.001 when compared to *Do gp.* using one-way ANOVA followed by Tukey-Kramer multiple comparison tests.
- BMI: body mass index; FPG: fasting plasma glucose; TC: total cholesterol; TAG: triacylglycerol; LDL-c: low-density lipoprotein-cholesterol; HDL-c: high-density lipoprotein-cholesterol.

Table 2:-Correlations among investigated parameters (№ 260)

Parameters	*r*	*Parameters*	*r*
Paraoxonase-1 Vs. disease duration	**-0.547 *****	**VCAM -1 Vs. disease duration**	**0.519 *****
Paraoxonase-1 Vs. FPG	**-0.553 *****	**VCAM -1 Vs. FPG**	**0.597 *****
Paraoxonase-1 Vs. A1c	**-0.544 *****	**VCAM -1 Vs. A1c**	**0.564 *****
Paraoxonase-1 Vs. TC	**-0.465 *****	**VCAM -1 Vs. TC**	**0.595 *****
Paraoxonase-1 Vs. TAG	**-0.621 *****	**VCAM -1 Vs. TAG**	**0.625 *****
Paraoxonase-1 Vs. HDL-c	**0.565 *****	**VCAM -1 Vs. HDL-c**	**-0.567 *****
Paraoxonase-1 Vs. LDL-c	**-0.593 *****	**VCAM -1 Vs. LDL-c**	**0.649 *****
Paraoxonase-1 Vs. MCP-1	**-0.514 *****	**VCAM -1 Vs. Lp (a)**	**0.484 *****
Paraoxonase-1 Vs. ICAM-1	**-0.516 *****	**Lp (a) Vs. disease duration**	**0.662 *****
Paraoxonase-1 Vs. VCAM -1	**-0.510 *****	**Lp (a) Vs. FPG**	**0.655 *****
Paraoxonase-1 Vs. Lp (a)	**-0.505 *****	**Lp (a) Vs. A1c**	**0.638 *****
MCP-1 Vs. disease duration	**0.504 *****	**Lp (a) Vs. TC**	**0.407 *****
MCP-1 Vs. FPG	**0.549 *****	**Lp (a) Vs. TAG**	**0.610 *****
MCP-1 Vs. A1c	**0.642 *****	**Lp (a) Vs. HDL-c**	**-0.639 *****
MCP-1 Vs. TC	**0.348 ****	**Lp (a) Vs. LDL-c**	**0.546 *****
MCP-1 Vs. TAG	**0.668 *****	**FPG Vs. A1C**	**0.803 *****
MCP-1 Vs. HDL-c	**-0.623 *****	**FPG Vs. TC**	**0.463 *****
MCP-1 Vs. LDL-c	**0.459 *****	**FPG Vs. TAG**	**0.638 *****
MCP-1 Vs. ICAM-1	**0.556 *****	**FPG Vs. HDL-c**	**-0.672 *****
MCP-1 Vs. VCAM -1	**0.501 *****	**FPG Vs. LDL-c**	**0.645 *****
MCP-1 Vs. Lp (a)	**0.588 *****	**A1C Vs. TC**	**0.425 *****
ICAM-1 Vs. disease duration	**0.560 *****	**A1C Vs. TAG**	**0.656 *****
ICAM-1 Vs. FPG	**0.585 *****	**A1C Vs. HDL-c**	**-0.687 *****
ICAM-1 Vs. A1c	**0.612 *****	**A1C Vs. LDL-c**	**0.580 *****
ICAM-1 Vs. TC	**0.385 *****	**TC Vs. TAG**	**0.671 *****
ICAM-1 Vs. TAG	**0.766 *****	**TC Vs. HDL-c**	**-0.396 *****
ICAM-1 Vs. HDL-c	**-0.620 *****	**TC Vs. LDL-c**	**0.782 *****
ICAM-1 Vs. LDL-c	**0.588 *****	**TAG Vs. HDL-c**	**-0.794 *****
ICAM-1 Vs. VCAM -1	**0.512 *****	**TAG Vs. LDL-c**	**0.680 *****
ICAM-1 Vs. Lp (a)	**0.539 *****	**HDL-c Vs. LDL-c**	**-0.566 *****

r: Pearson rank correlation coefficient assuming Gaussian distributions, **, ***: P value < 0.01, 0.001 respectively; FPG: fasting plasma glucose; TC: total cholesterol; TAG: triacylglycerol; LDL-c: low-density lipoprotein-cholesterol; HDL-c: high-density lipoprotein-cholesterol.

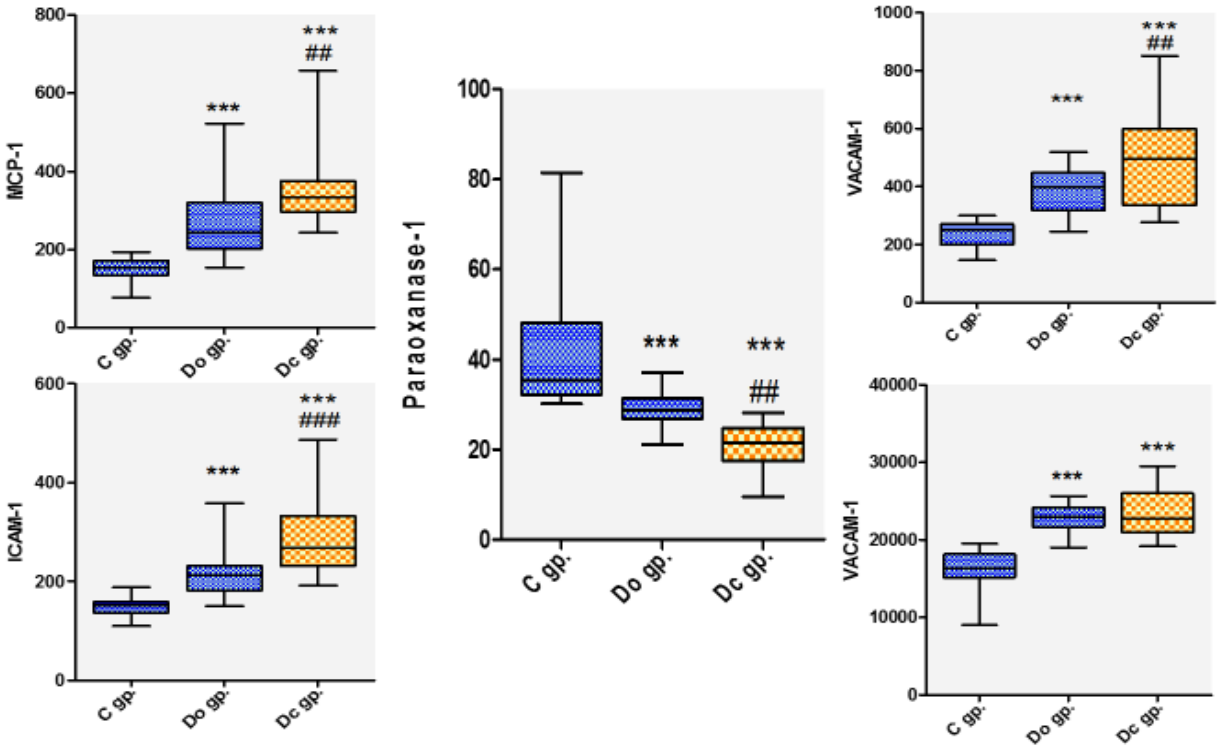

Fig. 1:-Serum levels of different measured parameters**.**

C gp., Do gp., Dc gp.: Healthy control group, Type 2 diabetic patients without complications, Type 2 diabetic patients with CVD. ***: $p < 0.001$ when compared to *C gp.* using one-way ANOVA followed by Tukey Kramer multiple comparison test. ##: $p < 0.01$, ###: $p < 0.001$ when compared to *Do gp.* using one-way ANOVA followed by Tukey-Kramer multiple comparison tests.

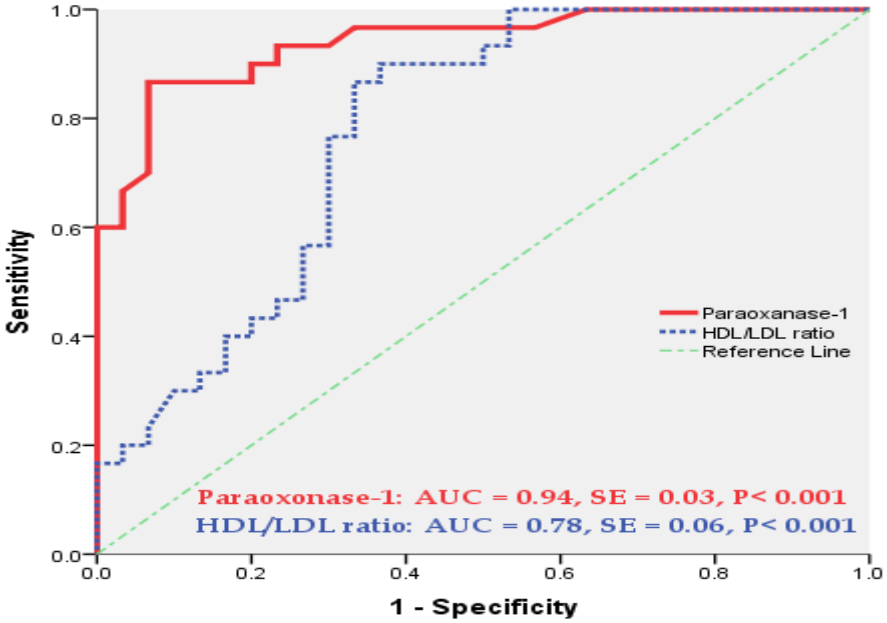

Fig. 2:-ROC analysis for anti-atherogenic risk assessment indices as predictors for CVD risk among diabetic cohorts.

AUC: Area under the curve, SE: standard error of mean. P: P value. Paraoxanase-1 was the closest to the top left-hand corner showing higher accuracy and bigger AUC (≈ 1).

Discussion:-

T2D is a multifactorial metabolic disorder characterized by chronic hyperglycemia caused by decreased production or sensitivity to insulin ***(Ozougwu et al., 2013)***. Chronic hyperglycemia results in a number of complications including CVD. Diabetic patients have more than double the risk of CVD-related mortality when compared to age-matched controls ***(Laakso, 2010)***. Despite the high prevalence of risk factors, no more than 25 % of the excess CVD risk in diabetes can be attributed to known risk factors, for example, smoking, hypertension, and atherogenic dyslipidemia ***(Pyorala et al., 1987)***. In T2D, vascular complications are mainly due to prolonged exposure to hyperglycemia clustering with other diseases such as hypertension and dyslipidemia and other risk factors including retinol binding protein-4 and hypoxia induced factor 1α ***(Paneni et al., 2013, Lambadiari et al., 2014, Li et al., 2014)***. In this sense, hyperglycemia is an important contributor to the onset of CVD complications. Among the biochemical alterations characteristic of hyperglycemia, the factors involved in causing atherosclerotic disease include the formation of advanced glycation end products, an increased polyol pathway flux and hexosamine pathway flux, and protein kinase C activation ***(Brownlee, 2001, Vlassara et al., 2002, Yan et al., 2003, Basta et al., 2004)***. All these molecular mechanisms reflect a single hyperglycemia induced process of superoxide overproduction by the mitochondrial electron transport chain. Hyperglycemia and an increased oxidative stress ***(Giuliano et al., 1996)*** thus lead to tissue damage via common pathways.

Results of this study showed that FPG and A1C levels were significantly higher in patients with T2D with CVD (*Dc gp*) compared to those without complications (*Do go*) and control group (*C gp*). Also, there is an increase in patients with T2D without complications (*Do go*) when compared to control group (*C gp*). These findings are in agreement with the previous study which suggest that the role of glucose in the development of atherosclerosis is thought to be mediated via several mechanisms; including platelet dysfunction, endothelial dysfunction, increased non-enzymatic glycation, alteration of lipids to a more atherogenic profile, and a procoagulant state ***(Horvit and Garber, 1998)***. Recently, the relative degrees of hyperglycemia, as measured by FPG levels or A1C at onset of T2D, were postulated to be important risk factors for the development of CHD ***(Lehto et al., 1997)*** and CVD ***(Kuusisto et al., 1994, Lehto et al., 1996)***.

Results of this study also showed that there is a significant increase in TC, TAG and LDL-c in T2D patients with CVD compared to those without complications and control group. Also, there is an increase in T2D patients without complications when compared to control group. On the other hand, there is a significant decrease in the HDL-c in T2D patients with CVD compared to control group and in T2D patients without complications when compared to control group and no significant decrease between T2D patients with CVD and T2D patients without complications. These results are in accordance with the previous study which observed a decrease of HDL-c level, and an increase of LDL-c, TAG and TC levels. ***Lapalu et al., 2007*** supported these results which appear to be associated with high risk of CVD development in diabetic patients. Rather, T2D by itself is considered a risk factor for several CVD, whose majority is descended from atherosclerosis ***(Kalmar et al., 2005)***. In ***1997, Lehto et al.*** reported that the lipid abnormalities could play a major role in the occurrence of the CVD accidents in diabetic patients. Effectively, the most prevalent diabetic CVD risk factor is dyslipidemia; it affects all lipid parameters, glycemic status, development of obesity and metabolic syndrome. All these factors will favor the installation of CVD in diabetic patients ***(Lehto et al., 1997)***.

Paraoxonase-1 (PON1) is an HDL-associated enzyme that hydrolyzes organophosphate compounds and fatty acid lactones ***(Mackness and Mackness, 2004, Costa et al., 2005, James, 2006, Gaidukov and Tawfik, 2007)***. PON1 activity has been inversely associated with CVD ***(Bhattacharyya et al., 2008)***, and PON1-deficient mice are more prone to develop atherosclerosis ***(Shih et al., 2000)***. PON1 activity was recently shown to be reduced in subjects with type 1 diabetes (T1D) and T2D ***(Deakin and James, 2004)*** and was inversely associated with glucose concentrations in subjects with T1D ***(Hofer et al., 2006)***. Results of this study showed that Paraoxonase-1 was significantly decreased in T2D patients with CVD compared to T2D patients without complications and control group. Also, there is a significant decrease in T2D patients without complications when compared to control group. These results came in accordance with the previous studies who reported that PON1 was found to be low in diabetic subjects without complications or with retinopathy ***(Mackness et al., 2000, Boemi et al., 2001)***. However, reports regarding PON1 activity in diabetic subjects with vascular complications are few. In ***2008, Mastorikou et***

al. demonstrated a significant decrease in PON1 activity in diabetic patients having complications compared to CVD patients without diabetes and attributed this decrease in PON1 activity to glycation of PON1 in diabetic subjects. Other study showed that the decreased PON-1 activity in diabetic patients besides the change in lipid parameters, metabolic abnormalities observed in T2D affect the reduction of the antioxidant capacity of HDL, and also the decreasing of PON-1 activity via changes in the activity of lipoprotein lipase leading to accelerate atherosclerosis process ***(Maritim et al., 2003)***. The low PON1 activity decreases the ability to prevent lipid peroxide formation with consequent acceleration of the oxidative stress. Overproduction of the reactive oxygen species in diabetic patients may be due to chronic hyperglycemia, hyperinsulinaemia, elevated free fatty acids and dyslipidemia ***(Gross et al., 2003, karabina et al., 2005)***.

Atherosclerosis is the result of an excessive proliferative and inflammatory response that includes smooth muscle cell migration and proliferation, inflammatory cell infiltration, neovascularization, production of extracellular matrix, and the accumulation of lipids ***(Ross et al., 1999)***. MCP-1 is involved in most of these processes ***(Reape and Groot, 1999)***. In the present study there is a significant increase in the MCP-1 concentration in T2D patients with CVD disease compared to T2D patients without complications and control group. Also, there is an increase in T2D patients without complications when compared to control group. These findings came in agreement with the previous studies which suggested that MCP-1 levels were significantly elevated in subjects with T2D as compared to non-diabetic subjects ***(Piemonti et al., 2003)***. Also, elevated MCP-1 was also found to correlate with atherosclerosis-associated complications, including ischemic stroke ***(Arakelyan et al., 2003)***, myocardial infarction ***(de Lemos et al., 2003)***, and CVD mortality ***(Piemonti et al., 2009)***. T2D patients may suffer from some forms of endothelial dysfunction which stimulates inflammation and increases levels of circulating soluble adhesion molecules (SAM) ***(Galen, 2002)***. Circulating levels of SAM are thought to reflect increased endothelial cell surface expression and high serum levels of SAM are considered markers of endothelial dysfunction ***(Galen, 2002, Rubio et al., 2007)***. Endothelial dysfunction seems to be the trigger in pathogenesis of atherogenesis and diabetes-associated vascular diseases and explains, at least in part, the enhanced progression of CVD in T2D.

In this study, circulating levels of ICAM-1 and VCAM-1 were significantly increased in T2D patients with CVD compared to patients without complications and control group. Also, there is an increase in T2D patients without complications when compared to control group. These findings came in agreement with previous studies which suggest that alterations in the vascular endothelium linked to diabetes may include elevated plasma levels of adhesion molecules and associated enhanced adhesion of monocytes to vascular endothelium, plus impairment of NO release and reduced NO responsiveness ***(Libby et al., 2002, Kim et al., 2006)***. The adhesion and migration of circulating macrophages are important in the initiation and progression of atherosclerotic disease. These processes are mediated largely by cellular-adhesion molecules ***(Ridker, 2001)***.

Lp (a) is an emerging CVD risk factor which is also associated with diabetes; it may act as a modulating factor of the vascular complications in T2D ***(Holmes et al., 2005, Okeoghene and Azenabor, 2011)***. The relationship between atherosclerosis and high concentrations of Lp (a) may be related to the homology of Lp (a) with plasminogen. Lp (a) inhibits the binding of plasminogen and stimulates the gene expression of the plasminogen activator inhibitor "in vitro". It has also been suggested that the interaction of Lp (a) with residues of glucosaminoglycans and proteoglycans from the arterial wall, and/or with macrophage recipients or scavengers, could play an important role in this association ***(Argraves et al., 1997)***. The similarity of Lp (a) to LDL and its ability to undergo oxidation are another reason why it has been implicated in atheroma development and has been suggested to be involved in foam cell formation, smooth cell proliferation, endothelial dysfunction, and vascular inflammation ***(Zeljkovic et al., 2009, Mansson et al., 2014)***. Several epidemiological studies have found clear association between Lp (a) and CVD and have suggested Lp (a) to be an independent risk factor for CVD ***(Marcovina et al., 1999, Danesh et al., 2000)***. In this study, authors found that Lp (a) level was significantly increased in both diabetic cohorts compared to control group. Surprisingly, we fail to find a significant alteration between the two diabetic cohorts regarding Lp (a) level although it's a major CVD risk factor. These results may support the previously published report which revealed that the effect of Lp(a) on CVD risk among diabetic patients might be different from that in the general population supporting the theory that diabetes status may attenuate the relation between Lp (a) and CVD risk ***(Qi et al., 2012)***. On the other hand, a recent study suggested that the plasma Lp (a) level may be a useful risk factor for the development of CVD in Korean patients with T2D ***(Min Kyong Moon, 2016)***.

Using ROC analysis, the clinical significance of Paraoxonase-1 as an anti-atherogenic marker was studied. The calculation of area under the ROC curve "AUC" describes two important issues; specificity and sensitivity, together they determine the overall diagnostic accuracy of an individual marker. The large the AUC is, the highest diagnostic accuracy will be. In the present study, analysis of ROC revealed that the highest diagnostic accuracy was achieved by Paraoxonase-1 (AUC ≈ 1). This study is one of the first studies examining Paraoxonase's diagnostic accuracy on Egyptian diabetic patients with a conclusion that Paraoxonase-1 could prove to be a marker for diagnosis and assessment of CVD in T2D.

Conclusion:-

We concluded that this panel of biomarkers can serve as new tools for the prediction of diabetic CVD complications in Egyptian T2D patients.

Research Highlights:-

From the present study serum Paraoxonase-1, MCP-1, ICAM-1, VCAM-1 and Lp (a) are most important powerful new tools for the diagnosis of CVD risk in T2D patients.

Also, the current study provided important data about some important biomarkers that could be used to predict the early signs of diabetic macrovascular complications. By using these biomarkers as novel markers of diabetic macrovascular complications, this will minimize the disease progression in the diabetic patients.

Limitations:-

The important limitations in this study that done in a small number of populations of patients.

Recommendations:-

Further studies of these biomarkers are needed to verify their predictive ability for the development of diabetic CVD and to determine if these could be used as biomarker of diabetic macrovascular complications and disease progression. Further studies should be done on large scale and upon other populations of this panel of proteins to reach the cutoff points of each marker in the diagnosis of diabetic CVD complications in the early stage.

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Author's Contribution and Competing Interests:-

The main contributors for this work were Dr.Heba Ibrahim Hamed and Dr. Ahmed Mohammed Abd El-Mohsin Akabawy under the guidance of Dr. Wafaa Salah Hegab and Dr. Naglaa Fawzy Mohamed. No competing interests were found.

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