

COMPARISON OF THE PREDICTIVE VALUE OF HSP60 TO OTHER MARKERS OF CARDIOVASCULAR DISEASE AMONG EGYPTIAN PATIENTS WITH TYPE 2 DIABETES

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ABSTRACT

Background: Although heat shock protein 60 (Hsp60) is implicated in the pathogenesis of atherosclerosis, its role in cardiovascular disease is uncertain. The objective of this study was investigate the level of Hsp60 in Egyptian patients with type 2 diabetes mellitus with / without cardiovascular risk factors and compare its level in these patients with some traditional biochemical parameters of cardiovascular complications to elucidate the potential value of Hsp60 in the development of T₂DM cardiovascular complications.

Methods and Results: 62 diabetic patients with different risks to cardiovascular disease (CVD) and 18 diabetic control patients were included. All groups were age and sex matched. Serum Hsp60 level was determined by Enzyme-linked immunoassay.

Results: Hsp60 levels were higher in diabetic patients with different risks to CVD complications compared to diabetic group, this increase was independent from other common diagnostic marker for CVD. Hsp60 showed the highest sensitivity compared to other traditional cardiac markers.

Conclusion: Presence of Hsp60 with hypertension, dyslipidemia and/or microalbuminuria in diabetic patients was cumulatively associated with greater risk of developing CVD complications. Hsp60 showed the superiority in sensitivity of compared to other traditional biochemical parameters so could serve as an early marker for diagnosis of CVD complications in diabetic patients before it becomes evident.

Keywords: Clinical, Heat-shock protein 60, Type 2 diabetes mellitus, Cardiovascular disease, Cystatin, Egyptian.

INTRODUCTION

Diabetes mellitus (DM) is a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood. The hyperglycemia is the result of a deficiency of insulin secretion or of resistance to the action of insulin in liver and muscle, or a combination of these. The chronic hyperglycemia produced due to diabetes leads to long-term damage to different organs including the heart, eyes, kidneys, nerves, and vascular system [1]. Diabetes is the eleventh most important cause of premature mortality in Egypt, and is responsible for 2.4% of all years of life lost. Similarly, diabetes is the sixth most important cause of disability burden in Egypt. [2].

In the year 2010 the total number of Egyptian diabetic patients is 4, 787000 patients and expected to be 8, 615000 patients by the year 2030 with annual increase 191, 000 [3].

Diabetes increases the risk that an individual will develop (CVD). Although the precise mechanisms through which diabetes increases the likelihood of atherosclerotic plaque formation are not completely defined, the association between the two is profound. CVD is the primary cause of death in people with either type 1 or type 2 diabetes [4, 5].

In fact, CVD accounts for the greatest component of health care expenditures in people with diabetes [5, 6].

Cardiac biomarkers may be defined as biological analytes that are detectable in the bloodstream at elevated levels during the continuum of cardiovascular diseases or immediately after myocardial damage [7].

Biomarkers for acute coronary syndrome are; biomarkers of atherosclerotic platelet activity for example Cytokines (Interleukins; IL1, IL6, IL8, IL10, IL18), myeloperoxidase (also marker of inflammation), acute phase reactants Fibrinogen, (AAS), C-reactive protein (CRP), Cystatin C, heat shock proteins and others [8].

There is a link between increased Cystatin C concentrations and impaired cardiovascular outcome, as reported in most of the studies, the important question is whether pathogenetic mechanisms other

than renal dysfunction could account for a high Cystatin C concentration and explain predictive value for future cardiovascular risk? Inflammation, associated with atherogenic changes, may be one mechanism associated with Cystatin C and cardiovascular risk [9, 10]. It has been suggested that high Cystatin C concentrations are directly related to both inflammation and atherosclerosis [11].

Heat Shock Proteins (Hsps) are "cellular lifeguards" that have antioxidant effects and anti-inflammatory action [12].

Hsps are also involved in the degradation of aged or damaged proteins. Under cellular stress, however, the expression of these stress proteins can be induced to prevent cellular damage. Hsps can be classified into six families, the small HSPs (sHsp), Hsp40, 60, 70, 90 and Hsp110, based on their molecular mass [13].

Members in the Hsp60 and Hsp70 families have been widely studied for their ability to stimulate innate and adaptive immunity as well as their abundance in cancer cells. The occurrence of these proteins is particularly important in diseases such as diabetes and CVD as, cells in the diabetic state are in stress due to hyperglycaemia, and endothelial inflammation is involved in the pathogenesis of cardiovascular diseases, a major complication in type 2 diabetes mellitus [14].

The aim of this work is to explore the circulating level of Hsp60 in Egyptian patients with type 2 diabetes mellitus with and without cardiovascular complications risk factors and compare its level in these patients with some traditional biochemical parameters of cardiovascular complications to clarify the potential value of Hsp60 in the development of T₂DM cardiovascular complications.

SUBJECTS AND METHODS

Subjects

The study comprised 80 Egyptian subjects (39 males and 41 females), recruited from the clinical pathology department at National institute for Diabetes and Endocrinology (NIDE). Patients were enrolled into the study after giving written informed consent. Before inclusion all the study subjects underwent careful physical examination, detailed history, and laboratory investigations to exclude any condition that may interfere with the studied parameters. Patients were divided into two main groups: 18 diabetic patients with no evidence of

microalbuminuria or cardiovascular complications act as control (D) and 62 diabetic patients with a with one or more risk factors for CVD which divided as: 8 diabetic with microalbuminuria (M), 9 hypertensive diabetic group (H), 12 Diabetic with dyslipidemia (L), 9 diabetic with hypertension and dyslipidemia (H+L), 8 diabetic with microalbuminuria and dyslipidemia (M+L), 9 diabetic with microalbuminuria and hypertension (M+H) and 7 diabetic with microalbuminuria, dyslipidemia and hypertension (M+H+L). Definition and selection of type 2 diabetes were done according to American Diabetes Association criteria [15].

All groups are age - and sex - matched. The study was approved by the research ethics committee of the General Organization for Teaching Hospitals and Institutes and the National Research Center (1/2012). Cairo, Egypt.

The characteristics of the patients are listed in table 1. Standing height and body weight were measured in light clothing without shoes.

A blood samples were drawn from all subjects after overnight fasting for the estimation of the investigated parameters. Samples handling, storage and preparation was done according to manufacturers' instructions.

Laboratory analyses

Fasting plasma glucose was measured using Dimension RxL analyzer (Dade Behring, Newark, DE) automated biochemistry analyzer and other serum biochemical parameters including triglycerides (TG), total cholesterol (TC), Serum high-density lipoprotein cholesterol (HDL-C), serum low-density lipoprotein cholesterol (LDL-C), serum creatinine and urea levels was measured using Spectrophotometer 1200 (UNICO Instruments.inc.USA). The A1c % was measured in whole blood with ion-exchange high-performance liquid chromatography using Bio-Rad D-10 system (Bio-Rad Laboratories, Hercules, CA). Serum Cystatin C and serum Hsp 60 levels were

determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits: Human Cystatin C ELISA kit (DRG @, Germany) and Hsp 60 ELISA kit (AssayPro @, USA). ELISA procedures were done by Automated ELISA system (The DiaSorin ETI-Max 3000 system, Italy) according to the manufacturer's instructions. The atherogenic ratios (TC/ HDL-C and LDL-C / HDL-C) were calculated. Microalbuminuria (expressed as A/C ratio mg/g creatinine) was measured in random urine samples taken from the patients using - ADVIA@ 1650 clinical chemistry system. Also the BMI was calculated as weight divided by squared height (in kilograms per square meter).

Statistics:

Statistics were done using GraphPad InStat tm (Graph software Inc., V 3.05, Ralf Stahlman, Purdue Univ.), to test significance of differences between groups. Appropriate graphs were plotted using GraphPad Prism 6 (Graphpad software Inc., V 6.00, USA). Correlation co-efficient was done using least square method. The accuracy indices were calculated according to Reed et al. [16]. P value less than 0.05 was considered statistically significant. Spearman's correlation analysis was used to analyze interrelationship between serum HSP60 levels and other clinical parameters. PSAW 18 formerly SPSS software (Statistical Package for the Social Sciences, version 18, SPSS Inc, Chicago, Ill, USA) was used to make ROC curve.

RESULTS

Clinical data of all subjects are shown in Table (1). Concerning age, sex and body mass index (BMI) (kg/m²) and using Tukey-Kramer multiple tests, no significant variation was verified between all the studied groups. Fasting blood level (FBGL) and glycated hemoglobin (A1c) showed no significance difference between the different studied groups.

Both serum TC and serum TG was significantly higher in L, H+L, M+L and M+H+L groups compared to D group (P< 0.05).

Table 1: Clinical and laboratory characteristics of the studied groups.

Markers	D (n=18)	M (n=8)	H (n=9)	L (n=12)	H+L (n=9)	M+L (n=8)	M+H (n=9)	M+H+L (n=7)
Gender	8 F/10M	5F/3M	5F/4M	5F/7M	5F/4M	5F/3M	4F/5M	4F/3M
BMI (kg/m ²)	28.717± 0.939	33.22± 2.27	34.556± 1.486	28.908±1.228	33.122±1.886	30.9±1.664	33.167±2.889	32.057± 1.996
Age (years)	47.667 ±2.016	48.375± 1.614	50.222± 1.913	50.222±2.715	48.667±2.703	46.125±1.563	49.333±3.424	49.424± 3.637
FBG (mg/dl)	176.44± 12.25	212.5± 19.82	229.4± 23.65	205.25±21.63	233.1±25.03	259.13±33.83	241±35.26	226.29± 37.8
Glycated Hb(%)	8.5± 0.513	8.813± 0.693	9.711± 0.815	9.067±0.746	9.544±0.67	10±0.694	9.467±1.055	8.9± 1.04
A/C ratio	17.056± 1.74	73.5± 23.38 a	14.522± 2.229 b	13.392±2.092 b	16.044±2.336 b	105.13±22.46 a, c, d, e	46.556±2.887 g	92.571± 23.4 a, c, d, e
Creatinine (mg/dl)	0.9888± 0.0359	1.358± 0.132 a	0.9744± 0.056 b	1.043±0.058	0.9889±0.0658	1.619±0.109 a, c, d, e	1.361±0.11 a, c, e	1.34± 0.086 a
Urea (mg/dl)	25.606± 1.758	24.733± 1.541	28.238± 3.187	24.426±2.245	27.118±1.046	26.595±4.179	27.287±3.055	39.796± 3.688 a, b, d, g
Total cholesterol (mg/dl)	172.94± 4.39	169.63± 6.25	165± 8.16	225.33±5.8 a, b, c	225.33±6.02 a, b, c	221.88±4.97 a, b, c, f	159.78±6.264 d, e	243.86± 11.6 a, b, c, f
Triglycerides (mg/dl)	166.29± 5.12	136.25± 10.21	162.22± 5.95	220.17±10.74 a, b	224.67±10.86 a, b	227.5±11.86 a, b	178.67±5.55	270.14± 45.4 a, b, c, f

F: Female, M: Male, FBG: Fasting blood glucose, BMI: Body mass index D: Diabetic group, M: Diabetic with microalbuminuria group, H: Diabetic hypertensive group L: Diabetic dyslipidemic group, H+L: Diabetic hypertensive & dyslipidemic group, M+L: Diabetic dyslipidemic with microalbuminuria group, M+H: Diabetic dyslipidemic with microalbuminuria group, M+H+L: Diabetic hypertensive & dyslipidemic with microalbuminuria group

The values are expressed as mean ± SEM.

a: compared to D group, **b:** compared to M group, **c:** compared to H group, **d:** compared to L group, **f:** compared to M+H group and **g:** compared to M+L group.

All results are considered significant at P<0.05

Using parametric ANOVA test followed by Tukey-Kramer multiple comparisons test.

Serum HDL-C was significantly lower and serum LDL-C was significantly higher in L, H+L, M+L and M+H+L groups compared to D group (P<0.001).

Regarding Kidney Function our results showed that M, M+L, M+H and M+H+L groups were significantly higher compared with D group (P<0.05, P<0.001, P<0.05 and P<0.05 respectively). M+L and M+H groups were significantly higher compared to H and H+L groups (P<0.001 and P<0.05 respectively), while, M+L group alone was significantly higher compared to L group (P<0.001), but H group was significantly lower compared to M

group (P<0.05). The level of serum urea in M+H+L group was significantly higher compared to D, M, L and M+L group (P<0.05).

Diagnostic tool for diabetic cardiovascular complications (Table 2)

Cardiovascular risk ratios (1&2) both were significantly higher in L, H+L, M+L and M+H+L groups compared D, M and H groups (P<0.001), and significantly higher in M+L and M+H+L groups compared to M+H group (P<0.001) but significantly lower in M+H compared with L and H+L groups (P<0.001).

Table 2: Diagnostic tools for diabetic cardiovascular complications

Markers	D (n=18)	M (n=8)	H (n=9)	L (n=12)	H+L (n=9)	M+L (n=8)	M+H (n=9)	M+H+L (n=7)
Cystatin C (ng/ml)	998.89±35.76	1071.8±84.4	1115.6±123.5	983.58±43.36	1133.7±93.6	1020.3±51.8	1229.7±109.9	1630.9±261.1
HSP 60 (ng/ml)	0.6557±0.0791	3.8728±0.499 a	3.1888±0.487 a	2.939±0.5655 a	2.2066±0.3108 a	2.1685±0.5985 a	3.697±0.585 a	1.2257±0.2685
TC/HDL (Ratio 1)	3.444±0.215	2.973±0.157	3.244±0.291	11.229±0.543 a, b, c	10.478±1.179 a, b, c	11.844±0.481 a, b, c, f	3.222±0.221 d, e	11.241±0.6948 a, b, c, f
LDL/HDL (Ratio 2)	1.857±0.138	1.49±0.122	1.62±0.251	7.638±0.346 a, b, c	7.322±0.992 a, b, c	8.363±0.61 a, b, c, f	1.471±0.168 d, e	7.743±0.598 a, b, c, f

F: Female, M:Male, FBG: Fasting blood glucose, BMI: Body mass index

D:Diabetic group, M:Diabetic with microalbuminuria group, H: Diabetic hypertensive group L: Diabetic dyslipidemic group, H+L:Diabetic hypertensive & dyslipidemic group, M+L:Diabetic dyslipidemic with microalbuminuria group, M+H: Diabetic dyslipidemic with microalbuminuria group, M+H+L: Diabetic hypertensive & dyslipidemic with microalbuminuria group

The values are expressed as mean±SEM.

a: compared to D group, b: compared to M group, c:compared to H group, d: compared to L group, f: compared to M+H group and g: compared to M+L group.

All results are considered significant at P<0.05

Using parametric ANOVA test followed by Tukey-Kramer multiple comparisons test.

The mean serum value of ACR in M, M+L and M+H+L groups were significantly higher compared to diabetic group (P<0.01, P<0.001 and P<0.001 respectively). M+L and M+H+L groups were significantly higher compared to H, L and H+L groups (P<0.001). In contrast H, L and H+L groups were significantly lower compared to M group (P<0.05, P<0.01 and P<0.05 respectively) and H group is significantly lower compared to M+L (P<0.05).

(P<0.001, P<0.05, P<0.05, P<0.001, P<0.01 and P<0.05 respectively).(figure 1)

Serum Hsp 60 showed significant increases in M, H, L, H+L, M+L and M+H groups compared to D group (P<0.01, P<0.05, P<0.01, P<0.01, P<0.05, P<0.05 and P<0.05 respectively).(figure 2)

The mean serum value of Cystatin C showed significant elevation in M+H+L group only compared to D, M, H, L, M+H and M+L groups

Regarding gender effect; Serum level of both Cystatin C and Hsp 60 showed no significant difference between males and females of the same group or between different studied groups.

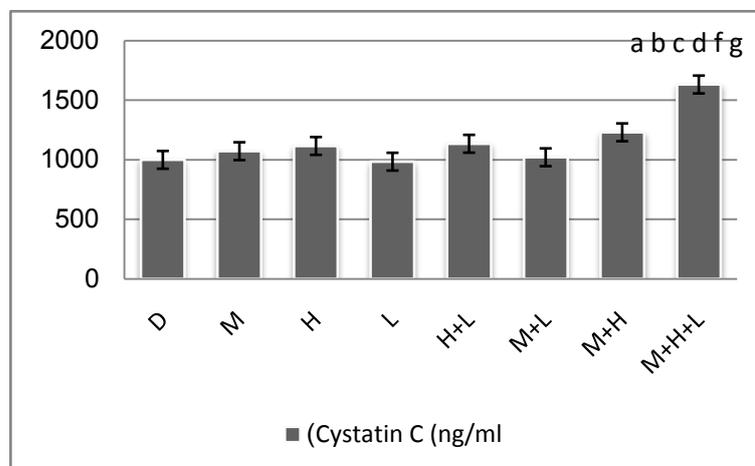


Fig. 1: Cystatin C (ng/ml) of D:Diabetic group, M:Diabetic with microalbuminuria group, H: Diabetic hypertensive group L: Diabetic dyslipidemic group, H+L:Diabetic hypertensive & dyslipidemic group, M+L:Diabetic dyslipidemic with microalbuminuria group, M+H: Diabetic dyslipidemic with microalbuminuria group, M+H+L: Diabetic hypertensive & dyslipidemic with microalbuminuria group

The values are expressed as mean±SEM.

a: compared to D group, b: compared to M group, c:compared to H group, d: compared to L group, f: compared to M+H group and g: compared to M+L group.

Results are considered significant at P<0.05 using parametric ANOVA test followed by Tukey-Kramer multiple comparisons test.

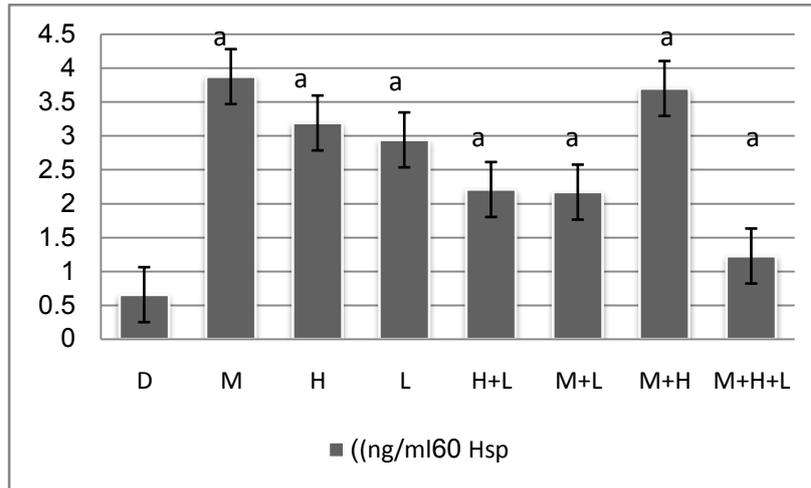


Fig. 2: HSP 60 (ng/ml) of D:Diabetic group, M:Diabetic with microalbuminuria group, H: Diabetic hypertensive group L: Diabetic dyslipidemic group, H+L:Diabetic hypertensive & dyslipidemic group, M+L:Diabetic dyslipidemic with microalbuminuria group, M+H: Diabetic dyslipidemic with microalbuminuria group, M+H+L: Diabetic hypertensive & dyslipidemic with microalbuminuria group

The values are expressed as mean±SEM.

a: compared to D group.

Results are considered significant at P<0.05 using parametric ANOVA test followed by Tukey-Kramer multiple comparisons test.

Correlations among investigated parameters

Simple linear regression analysis using Hsp 60 as dependent variable showed that, Hsp 60 showed a statistically significant direct

correlation with BMI (figure 3) and ACR (figure 4), while serum Cystatin C showed a statistically significant direct correlation with age and urea.

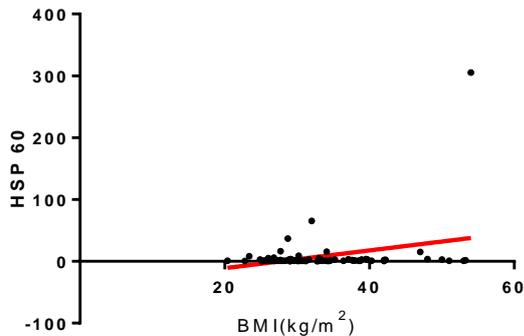


Fig. 3: Linear regression between Hsp 60 (ng/dl) and BMI (kg/m²)

r = 0.3085, p = 0.0054, no = 80

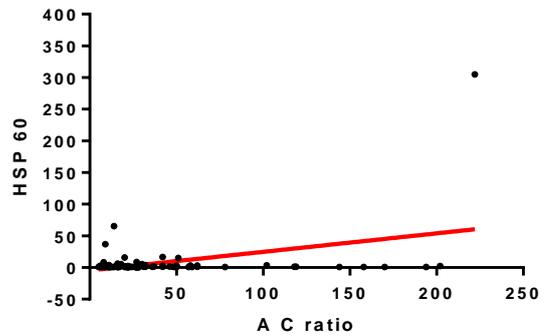


Fig. 4: Linear regression between Hsp 60 (ng/dl) and ACR (mg/g)

r = 0.397 p = 0.0003 no = 80

Diagnostic accuracy of CVD risk assessment indices

The diagnostic accuracy, sensitivity and specificity of Hsp60, Cystatin C, Ratio 1 and Ratio 2 were 93.6%, 62.7%, 64.5% and 65.5% respectively, and 53.3%, 66.7%, 76.4% and 72.2% respectively

(Table:3). Receiver operating characteristics (ROC) (figure:5) analysis for investigated parameters in studied patients (Table: 4), where quantitatively, area under the curve "AUC" is an overall measurement of accuracy, Hsp 60 Showed the biggest AUC ± SEM (0.716±0.08) than other traditional markers of CVD

Table 3: CV risk assessment accuracy indices

Markers	%Sn	%Sp	%PPV	%NPV	%A
Hsp 60	93.6	53.3	89.23	66.7	82.5
Cystatin C	62.7	66.7	86.1	35.3	61.25
Ratio 1	64.5	76.4	91	37.14	66.25
Ratio 2	65.5	72.2	88.8	38.23	66.25

Sn: Sensitivity, Sp: Specificity, PPV: Positive Predictive Value, NPV: Negative Predictive Value, A: Accuracy, Ratio 1: TC/HDL, Ratio 2: LDL/HDL.

Reference values (M ± SEM of control group): HSP 60= 0.6557± 0.07909 ng/ml, Cystatin C=998.89± 35.763 ng/ml, Ratio 1=3.444 ± 0.2152 and Ratio 2= 1.857±0.1376.

Data represented as percent.

Table 4: AUC of Hsp 60 and Cystatin C, Ratio 1 and 2

Variable	AUC± SEM	Asymptotic 95% CI	
		Lower bound	Upper bound
Hsp 60	0.716±0.08	0.56	0.873
Cystatin C	0.523±0.121	0.286	0.76
Ratio 1	0.151±0.053	0.48	0.254
Ratio 2	0.177±0.056	0.067	0.287

AUC: Area under the curve, SE: Standard error of means, 95% CI: 95% Confidence Interval. Ratio 1: TC/HDL, Ratio 2: LDL/HDL.

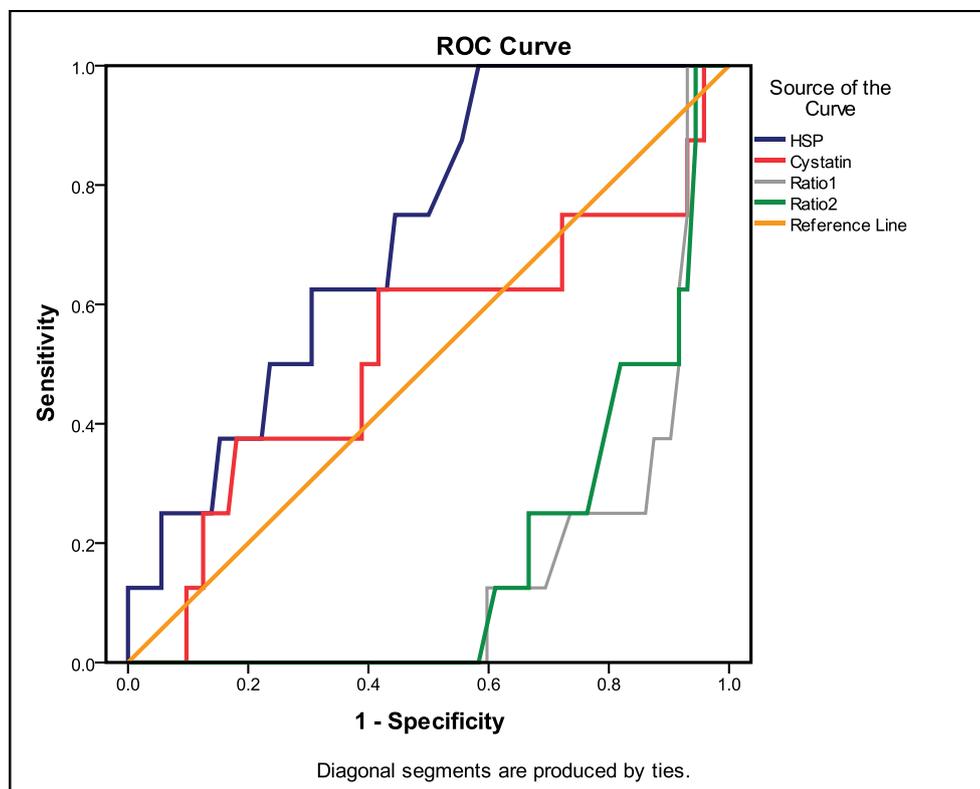


Fig. 5: ROC curve of diabetic cardiovascular markers

DISCUSSION

Four non-communicable diseases (NCDs) including: CVD, cancer, chronic respiratory disease, and diabetes were announced by World Health Organization (WHO) as the major causes of mortality in the world in 2008[17]. According to WHO prediction, in the next 10 years, mortality rate caused by NCDs will increase by 17 % with the highest mortality rate in the regions of Africa (27 %) and Eastern Mediterranean (EMRO, 25%). Fortunately more than 80 % of heart disease, stroke, and type 2 diabetes mellitus incidence and almost one third of cancers could be prevented with appropriate interventions to reduce the effect of risk factors [17].

The chronic hyperglycemia of diabetes caused by partial or complete insulin deficiency produces inadequate glucose control and is associated with long-term damage, dysfunction, and failure of different organs, and blood vessels [18].

Many patients with type 2 diabetes have hypertension, hyperinsulinemia and hyperlipidemia. All of these factors contribute to the long-term complications of diabetes, which include: Vascular disease (diabetic angiopathy), atherosclerosis, heart conditions and stroke: These cardiovascular disorders are the leading cause of death in people with diabetes [19].

Up to half of the events associated with cardiovascular diseases occur in asymptomatic individuals [20], emphasizing the need for true 'early' biomarkers. Microalbuminuria is considered the first sign and the best predictor of progression to renal failure and cardiovascular events. However, albuminuria has several

limitations. Therefore, earlier, more sensitive and specific biomarkers with greater predictability are needed [21].

Cystatin C levels an important risk factor for cardiovascular events [22]. High cystatin C levels reflect the duration and severity of other established risk factors. Reduced kidney function itself may be a risk factor for cardiovascular events so cystatin C as good marker for kidney dysfunction also is a link to cardiovascular complications. Cystatin C may have direct toxic effects that contribute to its association with risk of stroke and other cardiovascular events.

The stress protein Hsp60 is a nuclear-encoded protein that is found primarily in mitochondria. Although more commonly considered to be an intracellular molecule, it is now known that Hsp60 can be released from cells and that it is present in the peripheral blood of normal individuals [23, 24]. Furthermore, circulating Hsp60 has also been associated with the pathogenesis of atherosclerosis, diabetes, and cardiovascular disease [13, 25, 26]. And it has been reported that Hsp 60 levels were elevated in subjects suffering these diseases [24, 27, 28].

In the current study, Serum Hsp 60 was estimated in the samples of diabetic group with no evidence of microalbuminuria or cardiovascular complications, diabetic with various cardiovascular risk factors groups, and its biochemical effect was compared to other traditional markers and their correlation with cardiovascular complications in Egyptian diabetic patients with type 2 diabetes.

Measurement of FBGL and A1C is an integral part of the standard care for persons with DM and for research involved in assessment of glycemic control [29].

In the present study, serum Hsp60 level showed that individuals with microalbuminuria, hypertension, dyslipidemia, hypertension & dyslipidemia, microalbuminuria & dyslipidemia and microalbuminuria & hypertension groups was significantly higher compared to diabetic control group and this came in agreement with the results of Shamaei-Tousi et al who stated that a significantly higher proportion of patients with CVD had detectable circulating levels of Hsp60 compared with those without CVD [13].

Many researchers reported that there is an increasing body of data indicating that strict control of arterial pressure to levels, 140/90 mm Hg markedly reduces CVD morbidity and mortality and the development of end-stage renal disease in persons with type 2 diabetes [30, 31, 32]. In the Systolic Hypertension in the Elderly Program (SHEP) study, persons with type 2 diabetes derived more benefit from aggressive systolic blood pressure lowering in reduction of CVD than did those without diabetes [33]. Our results came also in agreement with Pockley et al, they have reported that Hsp60 levels are associated with early cardiovascular disease in individuals with borderline hypertension [24]. Regarding dyslipidaemia, reports showed that dyslipidaemia is associated with elevated total cholesterol, triglycerides and low level of high density lipoprotein (HDL), therefore, estimation of total cholesterol, triglycerides, and HDL has been used as the marker of dyslipidaemia, and dyslipidemia among the main risk factors for CVD, especially increase in LDL levels and decrease in HDL concentrations. [34, 35, 36].

Freedman et al showed that patients with advanced renal disease not only have a high incidence of cardiovascular disease (CVD), but CV morbidity and mortality is the leading cause of death in these subjects, particularly in those with diabetes. Also, albuminuria is associated with atherosclerosis even in subjects with relatively normal renal function [37].

The result of ACR in our study showed that mean serum ACR increased significantly in microalbuminuria, microalbuminuria with dyslipidemia and microalbuminuria with hypertension with dyslipidemia groups compared to diabetic control group, which came in agreement with Wachtell et al who mentioned that ACR levels below the cut point for microalbuminuria increased the risk for major cardiovascular events in individuals with or without diabetes [38].

Furthermore, in our study it was found that Ratio 1 and Ratio 2 were increased in dyslipidemia, hypertension with dyslipidemia, microalbuminuria with dyslipidemia and microalbuminuria with hypertension with dyslipidemia groups compared to diabetic control group. These results came in accordance with VinodMahato et al who reported that patients with HbA1c value >7.0% had significantly higher value of TC/HDL-C and LDL-C/HDL-C ratio as compared to the patients with HbA1c value ≤ 7.0% [39].

Our results showed that serum Cystatin C levels were elevated in microalbuminuria with hypertension with dyslipidemia group only compared to diabetic control group, and this come in agreement with Rafat et al who demonstrated that Serum cystatin C was significantly lower in the control group than in the nephropathy and CVD group [40].

In this study, studying the correlation between serum Hsp 60 and other CVD risk parameters showed that Hsp 60 was increased significantly in the serum of patients with risk to CVD and this increase was not correlated to age, sex or other biochemical risk factors (serum TC, TG, HDL-C, LDL-C and creatinine and Cystatin C) and this came in accordance with Mandal et al who showed that extracellular serum HSP60 is present in the blood of patients with CAD and that its concentration correlates with the severity of prevalent coronary atherosclerosis. Furthermore, they found this association to be independent of age, sex, CRP, and other established risk factors [41], and also Novo et al who reported that Hsp 60 levels are not correlated with sex [42], while serum HSP 60 in our study was found to be significantly correlated with body mass index (BMI) and this agreed with the results of Märker et al who proved that HSP 60 levels were higher in obese subjects than lean ones [43].

Comparing sensitivity and specificity of Hsp60 with other biochemical risk parameters of CVD, our data showed that sensitivity of HSP 60 as marker for prediction diabetic cardiovascular complications exceeding that of Ratio 1, Ratio 2 and Cystatin C and with comparable specificity to other markers, also Hsp60 showed higher areas under the ROC curve (AUC) compared to cystatin C, Ratio 1 and Ratio 2 indicates that it's superior in detection of early diabetic cardiovascular complications.

CONCLUSIONS

In summary, the present study showed increased serum level of Hsp60 in Egyptian diabetic patients with risk of CVD which may suggest the relationship between Hsp60 and development of CVD independently from any other common diagnostic marker for CVD and thus could serve as an early marker for diagnosis of CVD complications in diabetic patients before it becomes evident and also showed that the superiority of Hsp 60 compared to other traditional biochemical risk parameters. This need further studies on a more large scale for more confirmation and standardization to Hsp60.

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REFERENCES

1. Mealey BL, Ocampo GL. Diabetes mellitus and periodontal disease. *Periodontol* 2000; 44:127-53.
2. National Center of Health and Population (NICHIP). The Burden of Disease and Injury in Egypt (Mortality and Morbidity). 2004
3. Shaw JE, R.A. Sicree, P.Z. Zimmet. Diabetes Atlas Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice* 2010; 87: 4 – 14.
4. Laing SP Laing SP, Swerdlow AJ, Slater SD, Burden AC, Morris A, Waugh NR, Gatling W, Bingley PJ, Patterson CC. Mortality from heart disease in a cohort of 23, 000 patients with insulin-treated diabetes. *Diabetologia* 2003; 46:760–765.
5. Paterson AD Paterson AD, Rutledge BN, Cleary PA, Lachin JM, Crow RS. The effect of intensive diabetes treatment on resting heart rate in type 1 diabetes: the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study. *Diabetes Care* 2007; 30:2107–2112.
6. Hogan P, Dall T, Nikolov P. Economic costs of diabetes in the US in 2002. *Diabetes Care* 2003; 26:917–932.
7. McDonnell Barry, Stephen Hearty, Paul Leonard, Richard O'Kennedy. Review. Cardiac biomarkers and the case for point-of-care testing. *Clinical Biochemistry* 2009; 42 549–561.
8. Piñón Pablo, Juan Carlos Kaski. Inflammation, Atherosclerosis, and Cardiovascular Disease Risk: PAPP-A, Lp-PLA2, and Cystatin C. *New Insights or Redundant Information?* Review article. *Rev Esp Cardiol*. 2006; 59(3):247-58.
9. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, Curhan GC, and de Jong PE. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. *Kidney Int*. 2004; 65(4): 1416-1421.
10. Shlipak MG, Katz R, Cushman M, Sarnak MJ, Stehman-Breen C, Psaty BM, et al. Cystatin-C and inflammatory markers in the ambulatory elderly. *Am J Med* 2005; 118:1416.e25– e31.
11. Leung-Tack, J, Tavera C, Gensac MC, Martinez J, Colle A. Neutrophil chemotactic activity is modulated by human cystatin C, an inhibitor of cysteine proteases. *Exp. Cell. Res*. 1990; 188, 16.
12. Hooper, Philip L. M.D.1, Joanna J. Hooper. Loss of Defense against Stress: Diabetes and Heat Shock Proteins, B.A.2 *Diabetes Technology & Therapeutics*. 2005; Volume 7, Number 1.
13. Shamaei-Tousi A, Halcox JP, Henderson B. Stressing the obvious? Cell stress and cell stress proteins in cardiovascular disease. *Cardiovascular Research* 2007a; 74(1): 19-28.
14. An-Chi Tsuei, Ryan Dennis Martinus. Effects of metformin on human monocytic THP-1 cells – Implications for type 2 diabetes mellitus. 2011; *Cell stress and Chaperones*.

15. ADA. I. Classification and Diagnosis. *Diabetes Care* 2011; 34(suppl 1):S12
16. R. Reed et al. Choosing and Using Statistical Tests, Practical Skills in Bio-molecular Sciences, 2nd Edition, Pearson Education. 2003; p. 485.
17. Alwan A. 2008–2013 action plan for the global strategy for the prevention and control of noncommunicable diseases. Report World Health Organization 2008.
18. Akanksha Saklani, Versha Parcha, Ishan Dhulia, Deepak Kumar. Combined effect of coccinia indica (wight & arn) and salvadora oleoides (decne) on blood glucose level and other risk factors associated with type 2 diabetes mellitus in alloxan induced diabetic rats. *Int J Pharm Pharm Sci* 2012; Vol 4, suppl 4, 79-84.
19. Riaz S. Scientific Research and Essay 2009; Vol. 4 (5) pp. 367-373.
20. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non-HDL cholesterol, apolipoproteins A-I and B100, standard lipid measures, lipid ratios, and CRP as risk factors for cardiovascular disease in women. *J Am Med Assoc* 2005; 294:326–33.
21. Cohen-Bucay A, Viswanathan G. Urinary markers of glomerular injury in diabetic nephropathy. *Int. J. Nephrol.* 2012; 146987.
22. Ni L, Lu J, Bo Hou L, Yan T, Fan Q, Hui R, Cianflone K, Wang W, Wang W. Cystatin C, Associated With Hemorrhagic and Ischemic Stroke, Is a Strong Predictor of the Risk of Cardiovascular Events and Death in Chinese". *American Stroke association* 2007; 38:3287-3288.
23. Xu Q. Role of heat shock proteins in atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2002; 22: 1547–1559.
24. Pockley AG, Bulmer J, Hanks BM, and Wright BH. Identification of human heat shock protein 60 (Hsp60) and anti-Hsp60 antibodies in the peripheral circulation of normal individuals. *Cell Stress Chaperones.* 1999; 4: 29–35.
25. Xu Q, Schett G, Perschinka H et al. Serum soluble heat shock protein 60 is elevated in subjects with atherosclerosis in a general population. *Circulation.* 2000; 102:14–20.
26. Zhang X et al. Elevated heat shock protein 60 levels are associated with higher risk of coronary heart disease in Chinese. *Circulation.* 2008; 118:2687–2693.
27. Dasu MR, Devaraj S, Park S, Jialal I. Increased Toll-like receptor (TLR) activation and TLR ligands in recently diagnosed type 2 diabetic subjects. *Diabetes Care* 2010; 33:861–868.
28. Wu CT, Ou LS, Yeh KW, Lee WI, Huang JL. Serum heat shock protein 60 can predict remission of flare-up in juvenile idiopathic arthritis. *Clin Rheumatol* 2011;30; 959–965.
29. Sacks DB. Global harmonization of hemoglobin A1c. *Clin Chem* 2005; 51: 681-683.
30. Lindholm LH. The outcome of STOP-Hypertension-2 in relation to the 1999 WHO/ISH hypertension guidelines. *Blood Press Suppl.* 2000; 2:21–24.
31. Yusuf S et al. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients: the Heart Outcomes Prevention Evaluation Study Investigators. *N Engl J Med.* 2000; 342:145–153.
32. Sowers JR, Bakris GL. Antihypertensive therapy and the risk of type 2 diabetes mellitus. *N Engl J Med.* 2000; 342:969–970.
33. Curb JD et al. Effect of diuretic-based antihypertensive treatment of cardiovascular disease risk in older diabetic patients with isolated hypertension. *JAMA.* 1996; 276:1886–1892.
34. Labarthe DR. Coronary heart disease, In: Epidemiology and prevention of cardiovascular disease: a global challenge, Labarthe DR 2011; pp. 59-87, Jones and Bartlett, ISBN-13: 978-0-7637-4689-6, Canada.
35. Yusuf S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; 364: 937–52.
36. Akash Jain, Jasmine, Sunil Sharma, Vipin Saini. Hypolipidemic, hypoglycemic and antioxidant potential of saraca asoca thanolic leaves extract in streptozotocin induced- experimental diabetes. *Int J Pharm Pharm Sci* 2013; Vol 5, Suppl 1: 302-305.
37. Freedman BI et al. Relationship between albuminuria and cardiovascular disease in type 2 diabetes. *J Am Soc Nephrol* 2005; 16: 2156–2161.
38. Wachtell K et al. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: The LIFE Study. *Ann Intern Med* 2003; 139: 901 –906.
39. VinodMahato Ram et al. Association between glycaemic control and serum lipid profile in type 2 diabetic patients: Glycated haemoglobin as a dual biomarker. *Biomedical Research* 2011; 22 (3): 375-380.
40. Rafat H, Sameh H. Soror, Zeinab Hassan and Ashref Ismaail. Is Cystatin C a powerful Predictor of Cardiovascular Diseases in patients with type 2 Diabetes Mellitus? (Study on Egyptian patients). *Journal of Applied Pharmaceutical Science* 2011; 07: 54-58.
41. Mandal, A R Afzal, S J D Brecker, J Poloniecki, Q Xu, and M Jahangiri. Association of serum soluble heat shock protein 60 with toll-like receptor 4 polymorphism and severity of coronary artery disease. *Heart.* 2006; 92(5): 683–685.
42. Novo G, Francesco Cappello, Manfredi Rizzo, Giovanni Fazio, Sabrina Zambuto, Enza Tortorici, Antonella M. Gammazza, Simona Corrao, Giovanni Zummo, Everly C. De Macario, Alberto J. I. Macario, Pasquale Assennato, Salvatore Novo, Giovanni I. Volti. Hsp60 and heme oxygenase-1 (Hsp32) in acute myocardial infarction *Translational Research* 2011.
43. Märker T, Henrike Sell, Pia Zilleßen, Anja Glöde, Jennifer Kriebel, D. Margriet Ouwers, Piet Pattyn, Johannes Ruige, Susanne Famulla, Michael Roden, Jürgen Eckel and Christiane Habich. Heat Shock Protein 60 as a Mediator of Adipose Tissue Inflammation and Insulin Resistance. *Diabetes March* 2012; vol. 61(3): 615-625.