IMPACT OF GRANZYME B GENE EXPRESSION ON INFLAMMATION AND INSULIN RESISTANCE IN ATHEROSCLEROSIS

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ABSTRACT:

Background/Aim: Atherosclerosis is an inflammatory disease in which many immune cells are accumulated. Cytotoxic T lymphocytes, the dominant immune cells in advanced atherosclerotic lesions, exert their activity via proapoptotic protease granzyme B (GZB). However, increased GZB was detected in atherosclerotic lesions, its exact role and contribution toward atherosclerosis-related key regulatory processes yet unclear.

Method: GZB mRNA expression was quantified in peripheral blood of 15 apparently healthy controls and 58 atherosclerotic patients divided into 23 non-diabetic and 35 diabetic patients by Taqman RT-PCR. Serum high sensitivity C-reactive protein and insulin levels were estimated by ELISA.

Results: There is a significant 3-fold increase in GZB mRNA in atherosclerotic patients compared to control ($P<0.001$). Moreover, GZB mRNA levels were positively correlated with cardiovascular risk ratios, markers of inflammation and insulin resistance. Multiple regression analysis revealed that DM and current smoking were significant predictors for GZB gene expression.

Conclusions: GZB might have a role in inflammation and insulin resistance processes associated with atherosclerosis development and progression.

Key words:

Granzyme B, inflammation, insulin resistance, atherosclerosis

INTRODUCTION:

Atherosclerosis is a chronic inflammatory disease responsible for the majority of cardiovascular disease (CVD), one of major causes of death world-wide. Despite declines in CVD mortality over the past several decades, diabetes mellitus (DM) remains a key risk factor for CVD with a 2- to 4-fold higher risk of CVD, as well as an increased risk of mortality by up to 3-fold than those without DM **(Gregg, *et al.,* 2007; Preis *et al.,* 2009)**. Accumulating evidence showed that increased risk for CVD in type 2 DM is likely due to insulin resistance and underlying mechanisms of dyslipidemia, systemic inflammation and oxidative stress **(Gregg, *et al.,* 2007; Grundy, *et al.,* 1999)**.

Atherosclerosis was formerly identified as bland lipid-driven disease in which smooth muscle cells (SMCs) and endothelial cells are central effectors. However, substantial advances in basic and experimental investigations recognized many immune cells and mediators in atheroma, implicating inflammation as key regulatory process in all stages of atherosclerosis **(Libby, *et al.,* 2009; Mallat, *et al.,* 2009)**. Activated immune cells appear to play a major role in fibrous cap degradation and subsequent plaque rupture **(Ovchinnikova, *et al.,* 2009)**. Inflammatory response is indicated by circulating levels of biomarkers such as high sensitivity C-reactive protein (hsCRP) **(Schaub, *et al.,* 2012)**. It is worse mentioned that inflammatory response caused by immune cells such as macrophages and T-cells ultimately leads to insulin resistance, a hallmark of atherosclerosis and DM **(Shoelson, *et al.,* 2006)**.

Cytotoxic T lymphocytes (CTLs) account for about 50% of leucocytes infiltrate in advanced human atherosclerotic lesions, highlighting their critical role in atherogenesis **(Mallat, *et al.,* 2009; Kyaw, *et al.,* 2013)**. CTLs exert their cytotoxic activity mainly via structurally related superfamily of serine proteases known as granzymes **(Ewen, *et al.,* 2012)**. Granzyme B (GZB) is the most studied and best characterized member of granzymes family. Recent reports have shown that GZB is also expressed by various immune and non-immune cells such as neutrophils, mast cells, SMCs and macrophage **(Pardo, *et al.,* 2007; Kim, *et al.,* 2007; Choy, *et al.,* 2003)**. Both CTLs and macrophages are key inflammatory cells within atherosclerotic lesions **(Kyaw, *et al.,* 2013; Choy, *et al.,* 2003)**.

Until recently, intracellular role of GZB, specifically in the context of apoptosis via caspase-dependent and independent pathways was extensively studied. However, several lines of evidence now suggest a possible critical extracellular role of GZB in many chronic inflammatory conditions **(Boivin, *et al.,* 2009)**. GZB is released from activated CTLs into the extracellular spaces during target cell engagement, or even in absence of target cell engagement **(Prakash, *et al.,* 2008)**. Indeed, soluble forms of GZB were reported in plasma of normal individuals and increased in many inflammatory diseases such as unstable angina, acute myocardial infarction (AMI) **(Kondo, *et al.,* 2009)** and DM **(Ikemoto, *et al.,* 2009)**. However, increased GZB expression from leucocytes infiltrating atherosclerotic lesions together with SMCs was reported **(Kim, *et al.,* 2007)**, its expression in circulating leucocytes and its association with inflammatory response in atherosclerosis complicated with type 2 DM are not clearly investigated.

Hence, the current study was set out to assess the expression pattern of human GZB gene in peripheral blood of atherosclerotic coronary artery disease (CAD) patients. Furthermore, the relation of GZB to inflammatory activity (hsCRP) and insulin resistance as well as other metabolic (lipids), anthropometric and risk factors in atherosclerosis and type 2 DM-associated cardiovascular complications were being evaluated.

1   SUBJECTS AND METHODS:

1.1   Subjects:

A total of 73 males were enrolled in this study as follows; 58 atherosclerotic patients undergoing coronary artery bypass graft, being compared to 15 age-matched apparently healthy volunteers as the control group. Patients were subdivided into CAD/non-diabetic group; 23 patients and CAD/diabetic group; 35 patients. CAD patients were recruited from Cardiothroacic Surgery Department of Kobry El-Kobba Military Hospitals, Cairo, Egypt. A detailed family,

medical history and drug treatment were collected for all subjects. The general characteristics of the studied subjects are summarized in Table 1. Anthropometric parameters measured included body mass index (BMI). BMI was calculated as an index of the weight in kilograms divided by the square of the height in meters.

Table (1): Demographic data and biochemical parameters in control and CAD subgroups:

Parameters/Groups (n)	Control (15)	CAD/non-diabetic (23)	CAD/diabetic (35)
Age (years)	52.53 ± 0.83	53.17 ± 1.26	54.94 ± 0.78
Body mass index (kg/m^2)	26.42 ± 0.29	25.58 ± 0.36	26.69 ± 0.35
Current smoking, n(%)*	1(6.7%)	10 (43.5)[a]	17 (48.6)[a]
Hypertension, n(%)*	0(0%)	14 (60.9)[a]	26 (74..3)[a]
Triglycerides (mg%)	89.27 ± 6.95	129.65 ± 7.50[a]	156.863 ± 10.06[a,b]
Total cholesterol (mg%)	153.00 ± 3.86	175.78 ± 4.51[a]	191.66 ± 4.52[a,b]
HDL-C (mg%)	50.93 ± 2.08	37.52 ± 1.78[a]	36.74 ± 1.22[a]
LDL-C (mg%)	84.21 ± 2.98	112.33 ± 3.63[a]	123.54 ± 3.87[a,b]
TC/HDL-C	3.06 ± 0.13	4.89 ± 0.23[a]	5.42 ± 0.23[a]
LDL-C/HDL-C	1.70 ± 0.10	3.17 ± 0.20[a]	3.52 ± 0.18[a]
FBG (mg%)	91.93 ± 1.44	101.30 ± 2.07	176.71 ± 7.30[a,b]
HbA$_{1c}$ (%)	5.39 ± 0.07	5.78 ± 0.05	8.12 ± 0.24[a,b]
Fasting insulin[¶]	4.05 ± 0.44	10.95 ± 1.79[a]	10.15 ± 1.42[a]
HOMA-IR[¶]	0.93 ± 0.11	2.77 ± 0.48[a]	4.25 ± 0.61[a,b]
QUICKI	0.40 ± 0.01	0.35 ± 0.01[a]	0.32 ± 0.01[a,b]
WBCs count (10^3 cells/cm^3)	5.39 ± 0.25	7.52 ± 0.51[a]	6.89 ± 0.31[a]
Neutrophils count (% of WBCs)	51.00 ± 1.52	62.83 ± 1.81[a]	59.09 ± 1.55[a]
hsCRP (mg/L)[¶]	0.97 ± 0.25	4.70 ± 0.81[a]	12.54 ± 2.12[a,b]
GZB mRNA (fold expression)[¶]	1.00 ± 0.01	2.68 ± 0.33[a]	3.67 ± 0.32[a,b]

Data are expressed as mean $\pm$ SEM
a: significantly different from control at $p \leq 0.05$
b: significantly different from CVD/non-diabetic at $p \leq 0.05$
*: parameter analyzed by chi-square test
[¶]: parameter analyzed by Kruskal—Wallis test
CAD, coronary artery disease; TC, total cholesterol ; HDL-C, high density lipoprotein cholesterol; LDL-C; low density lipoprotein cholesterol; FBG, fasting blood glucose; GHbA1c, glycosylated hemoglobin; QUICKI, Quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment of insulin resistance; WBCs, white blood cells; hsCRP, high sensitivity C-reactive protein; GZB, granzyme B.

Subjects with history of type 1 DM, inflammatory diseases, autoimmune disorders, malignancy, hematological diseases, renal diseases or chronic liver disease were excluded from the study. Also, obese subjects with BMI ≥ 30 kg/m^2 were excluded. The study protocol was approved by ethical committees of Kobry El-Kobba Military Hospitals and Faculty of

Pharmacy, Ain Shams University, Cairo, Egypt. The study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki and a written informed consent was obtained from all participants.

1.2   Specimens:

Peripheral blood samples (10 mL) were collected from patients before the surgery and from controls after an overnight fasting. Blood samples were divided into 3 aliquots; the first aliquot of blood was collected on vacutainer tubes containing sodium fluoride for plasma preparation used for the assay of FBG. The second aliquot was collected on vacutainer tubes containing Na_2-EDTA for whole blood preparation used for the assay of glycosylated hemoglobin (HbA_{1c}%), complete blood count and RNA isolation within 1-3 hours after collection . The third aliquot was collected on plain vacutainer tubes for serum preparation used for the assay of lipids profile. The remaining serum and plasma were divided into several aliquots and stored at -60°C for subsequent assay.

1.3   Biochemical Analysis:

Serum biochemical parameters including: triglycerides, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and plasma FBG were determined using Roche/MODULAR automated analyzer (Roche Diagnostics). HbA_{1c}% was measured in whole blood with ion-exchange high-performance liquid chromatography using the Bio-Rad VARIANT II system (Bio-Rad Laboratories, Hercules, CA). Complete blood count was determined by Sysmex XT 2000i automated hematology analyzer (Sysmex Corporation, Japan).

Fasting serum insulin levels were determined by enzyme linked immunosorbent assay (ELISA) kit supplied by Immunospec Inc, USA. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from fasting insulin and FBG by the following equation: HOMA-IR= fasting insulin (µU/mL) $\times$ FBG (mg/dL)/405 (**Matthews, *et al.,* 1985**). The quantitative insulin sensitivity check index (QUICKI) was calculated by the following equation: QUICKI= 1/[log(Fasting insulin) + log(FBG)] (**Katz, *et al.,* 2000**). Serum hsCRP levels were estimated by ELISA technique using commercially available ELISA kit supplied by Monobind Inc, USA. All ELISA procedures were done by Hyprep automated ELISA system (Hyperion Inc, Miami, FL) according to the manufacturer's instructions.

1.4   RNA Isolation and cDNA synthesis:

Total RNA was extracted from whole blood by QIAamp RNA Blood Mini kit (Qiagen, Germany). NanoDrop 2000 (Thermo Scientific, USA) was used to determine RNA concentration and purity. Total RNA (500 ng) was reversely transcribed to cDNA with High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, USA). cDNA (final volume of 20 µL) was stored at -20°C for subsequent quantification of human GZB gene.

1.5   Real-Time Quantitative PCR:

Real-time PCR analysis was performed on Applied Biosystems 7500 Real-Time PCR instrument using Taqman® Universal Master Mix II (Applied Biosystems) containing AmpErase uracil-N-glycosylase (UNG). Primers and probe Taqman® gene expression assays for human GZB and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; housekeeping gene) were supplied by Applied Biosystems (Hs0015543355_m1 and Hs02758991_g1, respectively). Taqman real-time PCR was performed using 50 ng cDNA template, 1 µL Taqman® gene

expression assay and 10 µL Taqman® universal master mix in final volume of 20 µL. The reaction PCR protocol used involved heating for 2 min at 50°C for UNG activation to remove any uracil incorporated into the cDNA then heating for 10 min at 95°C for polymerase activation, followed by 40 cycles of amplification (15 s at 95°C and 1 min at 60°C). Relative quantification of target gene in samples was expressed as fold-induction of the target gene compared to the reference gene according to the formula $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct = $ (Ct target gene − Ct GAPDH)$_{sample}$ − (Ct target gene − Ct GAPDH)$_{control}$ and Ct is the threshold cycle. A negative control using nuclease-free water instead of cDNA template was included in each experiment.

1.6 Statistical Analysis:

Data were expressed as mean ± SEM. Comparison between groups was done using one-way ANOVA for parametric variables and Kruskal Wallis test for nonparametric data. Correlations between markers were analyzed with Ranked Spearman test. Finally, multiple stepwise linear regression analysis was performed to examine factors significantly associated with GZB gene as a dependent variable. Statistical significance was accepted when the *p* values were ≤ 0.05. All statistical analysis was performed by IBM statistical package for social sciences (SPSS) statistics version 16.0.

RESULTS:

1.7 Group comparisons of the studied population:

General and laboratories parameters of the studied groups are summarized in Table (1). There was no significant difference between groups in age and BMI. Regarding lipids profile, both CAD subgroups showed significant increase in triglycerides, TC, LDL-C and cardiovascular risk ratios (TC/HDL-C and LDL-C/HDL-C) as well as significant decrease in HDL-C levels in comparison to control group ($P \leq 0.01$). Moreover, triglycerides, TC and LDL-C were significantly elevated in CAD/diabetic patients compared to CAD/non-diabetic patients ($P \leq 0.05$).

Concerning FBG and HbA$_{1c}$%, only CAD/diabetic but not CAD/non-diabetic group showed significant elevation compared to the control group ($P \leq 0.0001$). While both CAD subgroups showed significant elevation in fasting insulin levels and insulin resistance index; HOMA-IR as well as significant reduction in QUICKI index as compared to the control group ($P < 0.01$). This significance remained between both CAD subgroups in HOMA-IR and QUICKI index ($P < 0.05$). As revealed in Table (1) and Figure (1), levels of serum hsCRP and GZB mRNA showed significant elevation in both CAD subgroups as compared to control group ($P < 0.0001$). Additionally, serum hsCRP and GZB mRNA levels tended to be significantly higher in CAD/diabetic than CAD/non-diabetic ($P < 0.01$ and 0.05, respectively).
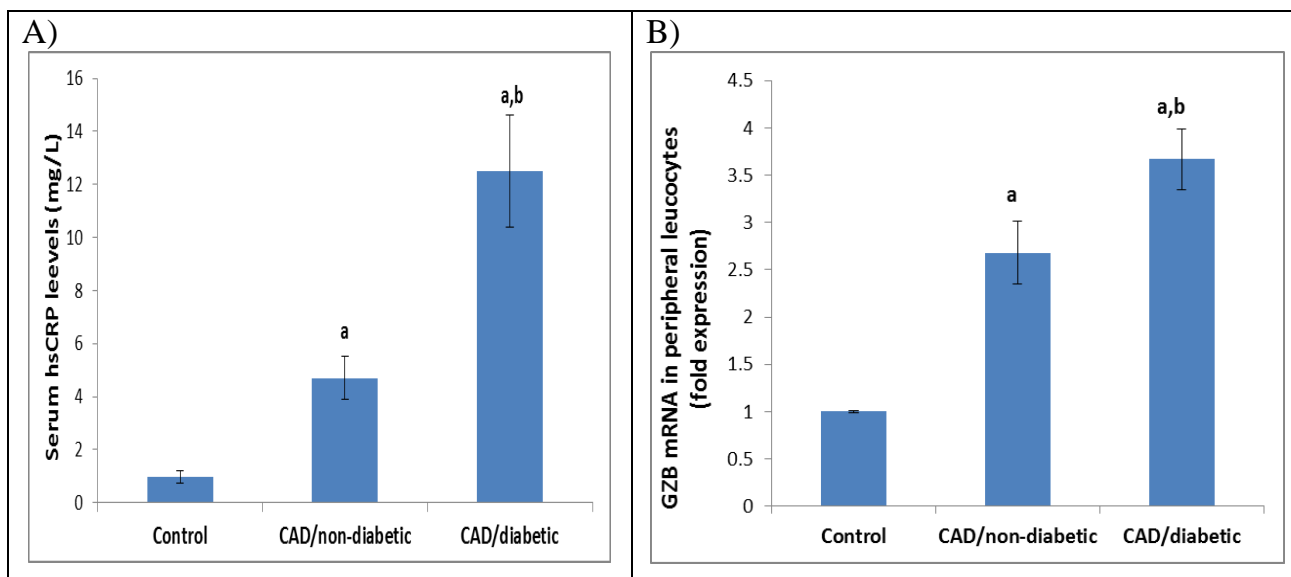
Figure (1): (A) Serum hsCRP levels **(B)** GZB mRNA expression levels in peripheral leucocytes. Data are expressed as mean ± SEM and analyzed by Kruskal Wallis test. a; significantly different from control at $P \leq 0.001$, b; significantly different from CAD/non-diabetic at $P \leq 0.05$.

1.8   Correlation between anthropometric and metabolic parameters and GZB mRNA:

As shown in Table (2) and Figure (2), investigation of bivariate correlation between GZB mRNA and anthropometric and metabolic parameters revealed that GZB mRNA was positively correlated with triglycerides, TC and LDL-C (r= 0.342, 0.426 and 0.515 at $P< 0.01$, respectively) as well as cardiovascular risk ratios (TC/HDL-C and LDL-C/HDL-C) (r= 0.475 and 0.463 at $P< 0.001$, respectively) Also, GZB mRNA was positively correlated with FBG, $HbA_{1c}\%$, and HOMA-IR (r= 0.564, 0.496 and 0.447 at $P<0.001$, respectively).   Regarding markers of inflammation, GZB mRNA showed significant positive association with hsCRP and neutrophils count (r=0.479 and 0.305 at $P<0.01$, respectively) as well as white blood cells (WBCs) count (r=0.265 at $P<0.05$). Also, GZB mRNA was negatively correlated with HDL-C and QUICKI index (r= - 0.360 and -0.427 at $P<0.01$ and 0.001, respectively).

Table (2): Simple correlations of blood GZB mRNA levels with other metabolic parameters:

Parameters	Blood GZB mRNA	
	r	*P*
Triglycerides	0.342	0.003
Total cholesterol	0.426	< 0.0001
HDL-C	- 0.360	0.002
LDL-C	0.515	< 0.0001
TC/HDL-C	0.475	< 0.0001
LDL-C/HDL-C	0.463	< 0.0001
FBG	0.564	< 0.0001
$HbA_{1c}\%$	0.496	< 0.0001
HOMA-IR	0.447	< 0.0001
QUICKI	- 0.427	< 0.0001
hsCRP	0.479	< 0.0001
WBCs count	0.265	0.024
Neutrophils count	0.305	0.009

GZB, granzyme B; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C; low density lipoprotein cholesterol; FBG, fasting blood glucose; GHbA1c, glycosylated hemoglobin; QUICKI, Quantitative insulin sensitivity check index; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; WBCs, white blood cells
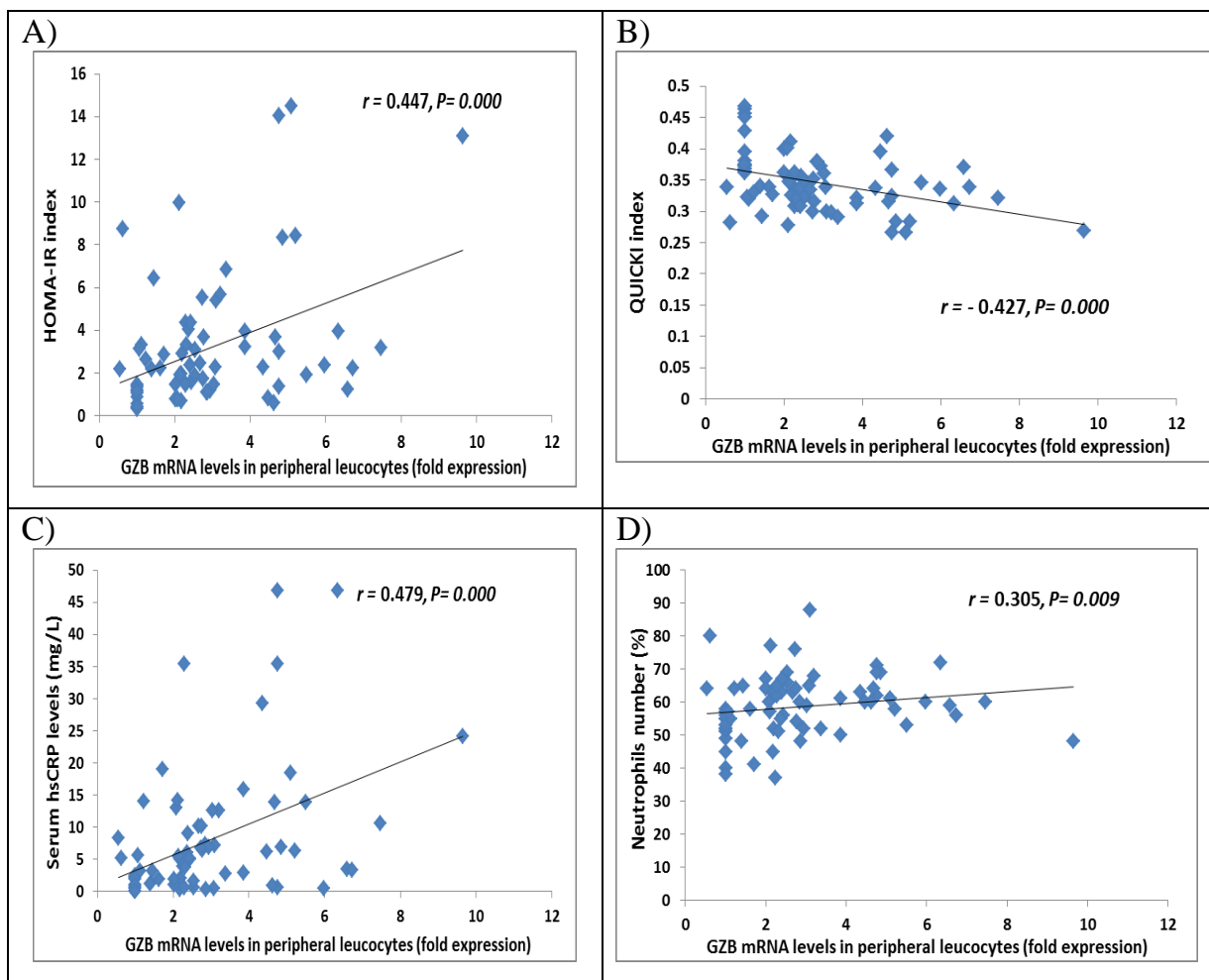
Figure (2): Correlation of GZB mRNA levels with different parameters **(A)** Correlation with HOMA-IR index **(B)** correlation with QUICKI index **(C)** Correlation with serum hsCRP levels **(D)** Correlation with neutrophils number.

1.9   Multiple stepwise linear regression analysis for GZB mRNA as a dependent variable:

We conducted simple linear regression, and then multiple stepwise linear regression analysis (Table 3) to identify predicting factors significantly associated with GZB gene expression levels. We set GZB mRNA levels as a dependent variable and age, BMI, current smoking, hypertension, DM, hypercholesterolemia, HOMA-IR, hsCRP and patient history of previous AMI as independent variables. Simple regression analysis revealed that hypertension, smoking, DM, hypercholesterolemia, HOMA-IR and hsCRP are positively predicting GZB mRNA levels. While multiple stepwise linear regression analysis demonstrated that DM and current smoking are predictors for GZB mRNA levels (*β= 0.446 and .258 at P* = 0.000 and 0.017, respectively).

Table (3): Univariate and multiple stepwise linear regression analysis using GZB mRNA as dependent variables:

Parameters	GZB mRNA¶			
	Simple univariate		Multiple stepwise	
	ß	*P*	*ß*	*P*
Age	0.162	0.171	…..	…..
Body mass index	0.167	0.159	…..	…..
Current smoking	0.375	0.001	0.258	0.017
Hypertension	0.384	0.001	…..	…..
Diabetes mellitus	0.532	0.000	0.446	0.000
Hypercholesterolemia	0.294	0.011	…..	…..
HOMA-IR¶	0.418	0.000	…..	…..
hsCRP¶	0.348	0.004	…..	…..
History of previous AMI	0.250	0.034	…..	…..

GZB, granzyme B; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high sensitivity C-reactive protein; AMI, acute myocardial infarction

¶ Log-transformed before analysis; R^2 for stepwise regression model was 30.4%

DISCUSSION:

Atherosclerosis is an inflammatory disease characterized by accumulation of lipids and many cells as SMCs, macrophages and T lymphocytes as well as extracellular matrix proteins (**Libby,** *et al.,* **2009; Libby,** *et al.,* **2002**). It is widely accepted that inflammation and insulin resistance are important players in atherosclerosis and type 2 DM (**Libby,** *et al.,* **2009; Donath and Shoelson, 2011**). Because of high mortality rate of CVD, identifying new pathways implicated in inflammation and insulin resistance is great for understanding this disease and provides hope for the development of novel therapeutic strategies aiming to reduce atherosclerosis-related mortality. Our interest in GZB is based on several points.

GZB is a serine protease produced by CTLs and macrophages. Both are central cells contributing to development and complications of human atherosclerotic plaques (**Kyaw,** *et al.,* **2013; Choy,** *et al.,* **2003**). Besides well recognized apoptotic role of GZB via perforin-dependent pathways, its extracellular role now received increased interest. Recent work by our co-workers has demonstrated elevated circulating levels of GZB in acute coronary syndrome (**El-Mesallamy,** *et al.,* **2013**), obesity and type 2 DM (**El-Mesallamy,** *et al.,* **2013**), suggesting important role of this protease in these inflammatory conditions. However, studies revealed increased expression of GZB in lipid rich areas of atherosclerotic plaques (**Kim,** *et al.,* **2007; Choy,** *et al.,* **2003**), the related mechanisms involved in atherosclerosis needs further investigation. Accordingly, our study evaluated the changes in gene expression of GZB in atherosclerotic patients with and without type 2 DM. We also focused on investigating its association with other metabolic parameters.

The current study demonstrated increased expression of GZB mRNA in peripheral blood of CAD patients with or without DM in comparison to control group. This finding comes in

accordance with studies revealed increased circulating levels of GZB after AMI (**Kondo,** *et al.,* **2009**), stable and unstable angina pectoris (**Tsuru,** *et al.,* **2008**) as well as angina complicated with chronic kidney disease (**Ikemoto,** *et al.,* **2009**). Most of studies focused on GZB-mediated apoptosis and their contribution to atherosclerosis pathogenesis. While in our study, we studied GZB contribution to inflammation and other metabolic disturbances associated with atherogenesis. Correlation analysis showed that GZB mRNA levels were positively correlated with triglycerides, TC and LDL-C and negatively correlated with HDL-C, indicating its relation to lipid disorders involved in atherosclerosis development and progression.

Indeed, GZB mRNA is significantly associated with hsCRP, an important marker of chronic low-grade inflammation. Evidence from experimental and clinical studies has been accumulating that CRP is not just a sensitive markers of systemic inflammation but also an amplifier of it. It is widely accepted that hsCRP is an independent risk for CVD and could participate directly in the pathogenesis of atherosclerosis through a variety of mechanisms (**Koenig and Khuseyinova, 2007; Yeh,** *et al.,* **2001**). A reasonable explanation for correlation between GZB and hsCRP could be extracellular role of GZB in proteolysis of extracellular matrix proteins such as vitronectin, fibrillin and fibronectin and subsequent induction of macrophages to secrete proinflammatory cytokines (**Buzza,** *et al.,* **2005; Chamberlain and Granville, 2007**). Moreover, GZB may influence inflammation though direct activation of other cytokines as interleukin (IL)-18 and IL-1β by proteolytic cleavage of their inactive precursors at the same residue cleaved by caspase-1 (**Omoto,** *et al.,* **2010**). Furthermore, contribution of GZB to inflammation is supported by its positive correlation to WBCs count and neutrophils count. Both are well established markers of systemic inflammation and important players in atherogenesis (**Ortega,** *et al.,* **2012; Soehnlein, 2012**). High WBCs is not only a marker of activated immune system but also found to be associated with all stages of atherosclerosis independently of traditional risk factors (**Ortega,** *et al.,* **2012**).

With regards to insulin resistance, we demonstrated that GZB mRNA levels were positively correlated with FBG, HbA_{1c}% and HOMA-IR as well as negatively associated with QUICKI index. Previously hyperglycemia was claimed to cause activation of CTLs (**Stentz FB and Kitabchi, 2003**) that could eventually lead to an increased release of GZB. Additionally, a recent study in our lab has shown that plasma GZB levels were elevated in patients with type 2 DM as compared to non-diabetic subjects and positively correlated with circulating insulin receptor α-subunit levels (**El Mesallamy,** *et al.,* **2013**). The latter finding might support the new hypothesis of insulin resistance which based on cleavage of insulin receptor by proteases as serine proteases and matrix metalloproteinases (**DeLano,** *et al.,* **2010**). Association between GZB and insulin resistance could be also attributed to GZB-dependent induction of potential proinflammatory cytokines as IL-1α and IL-18 (**Omoto,** *et al.,* **2010; Afonina,** *et al.,* **2011**). It is worse mentioning that association between GZB mRNA and WBCs count might support the possible role of GZB in insulin resistance as high WBCs was found to be a predictor for worsening in insulin sensitivity even in absence of adiposity (**Vozarova,** *et al.,* **2002**).

Moreover, multiple regression analysis demonstrated that DM is significantly associated with GZB mRNA levels. This comes in accordance with a study of Ikemoto and coworkers who found the contributory role of DM to plasma GZB levels in chronic renal failure patients (**Ikemoto,** *et al.,* **2009**). This could be attributed to increased cellular protein kinase C activity in diabetic patients and subsequent upregulation of GZB expression (**Tsuru,** *et al.,* **2008**). This is supported by elevation of GZB mRNA levels observed in CAD/diabetic patients as compared to

non-diabetics. Therefore, GZB might be a candidate molecule involved in atherosclerosis with and without DM.

In conclusion, we demonstrated significant increase in GZB gene expression from circulating leucocytes in atherosclerotic patients with and without type 2 DM. Moreover, GZB gene is significantly associated with inflammation and insulin resistance, hallmarks of atherosclerosis. Therefore, we propose that the modulation of GZB signaling may attenuate atherosclerosis progression and reduce its complications associated with type 2 DM.

Conflict of Interests

The authors declare that there is no conflict of interests.

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تأثير التعبير الجيني للجرانزيوم ب على الالتهاب ومقاومة الانسولين في تصلب الشرايين

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الخلفية / الهدف: تصلب الشرايين هو مرض التهابي يتميز بتجمع العديد من الخلايا المناعية. وتعتبر الخلايا تي الليمفاوية السامة للخلايا هي الخلايا المناعية المسيطرة في آفات تصلب الشرايين المتقدمة. وتمارس هذه الخلايا نشاطها عبر البروتين المسبب لموت الخلايا المبرمج الجرانزيوم ب. وبالرغم من الكشف عن زيادة هذا البروتين في آفات تصلب الشرايين، الا أن دوره المحدد ومساهمته اتجاه العمليات التنظيمية الرئيسية المتعلقة بتصلب الشرايين حتى الآن غير واضحة.

طرق البحث: تم قياس التعبير الجيني للجرانزيوم ب في كريات الدم البيضاء في عدد 15 من المتطوعين الأصحاء يمثلون المجموعة الضابطة وعدد 58 مريضا بتصلب الشرايين مقسمين الى 23 من غير المصابين بمرض البول السكري من النوع الثاني و35 من المصابين بمرض البول السكري من النوع الثاني باستخدام تقنية تفاعل البلمرة المتسلسل اللحظي. كما تم قياس مستوي البروتين عالى الحساسية التفاعلي سي والانسولين باستخدام تقنية معايرة الإنزيم المرتبط الممتز المناعي.

النتائج: وجدنا أن مستوى التعبير الجيني للجرانزيوم ب مرتفعا بما يقارب الثلاثة أضعاف في جميع مرضى تصلب الشرايين بالمقارنة مع المجموعة الضابطة من الأصحاء. علاوة على ذلك، كانت مستويات التعبير الجيني للجرانزيم ب مرتبطة بشكل إيجابي مع نسب مخاطر القلب والأوعية الدموية، وعلامات الالتهاب ومقاومة الانسولين. وكشف تحليل الانحدار المتعدد أن مرض البول السكري من النوع الثاني والتدخين الحالي من العوامل التى تتنبىء بالتعبير الجيني للجرانزيوم ب.

الاستنتاجات : قد يكون للجرانزيوم ب دور مهم في عمليات الالتهاب و مقاومة الأنسولين المرتبطة بتنمية وتقدم مرض تصلب الشرايين .

الكلمات الدالة: الجرانزيوم ب، الالتهاب، مقاومة الأنسولين، وتصلب الشرايين