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Glutathion, Glutathion Reductase and Gama-glutamyl Transferase Biomarkers for Type 2 Diabetes Mellitus and Coronary Heart Disease

Zainab AA Al-Shamma[1] *MSc*, **Hedef D El-Yassin**[2] *PhD*

[1]Dept. of Clinical Pharmacy and Therapeutics, [2]Dept. of Physiological Chemistry, College of Medicine, Baghdad University

Abstract

Background Hypercholesterolemia, one of the major risk factors of atherosclerosis, is a major health problem in the world that enhances the free radical generation in various ways. The level of antioxidants was decreased in hypercholesterolemic patients. This depletion of antioxidants may increase in type 2 diabetic patients with hypercholesterolemia, which also may increase the risk of complications from the most common form of diabetes mellitus.

Objective To evaluate serum reduced glutathione and glutathione reductase as an antioxidant, and gamma-glutamyl transferase as a marker of oxidative stress in both hypercholesterolemic and diabetic-hypercholesterolemic patients.

Methods The study involved 33 diabetic hypercholesterolemic patients, 37 hypercholesterolemic and 54 healthy control subjects. Ten ml of blood were collected from each patient and normal control subject after an overnight fast for the measurement of gluathion (GSH) glutathione reductase (GR) and gamma-glutamyl transferease (GGT), glucose, lipid profile, urea, creatinine and glycated Hb (HbA1c). The last was for the diabetics only.

Result Showed a significant decrease in GSH and GR in diabetic-hypercholesterolemic patients compared with hypercholseterolemic patients and a significant increase in GGT in both groups compared with controls. There was a negative correlation between cholesterol with GR in both groups of patients involved in this study and a negative correlation between HbA1c and each of GSH and GR in the diabetic-hypercholesterolemic patients.

Conclusions High levels of oxidative stress and reduced antioxidants in people with coronary heart disease, previously thought to be markers of the heart condition, could also, indicate a condition of glucose abnormality, such as overt type 2 diabetes.

Key words hypercholestermia, type 2 diabetes, antioxidants, oxidative stress, GGT.

Introduction:

Oxygen free radicals (OFRs) play a significant role in the pathogenesis of many diseases like atherosclerosis, cancer, neuro-degeneration and inflammation. Their production may be greatly enhanced by exogenous factors like environment pollutants, drugs, radiation and pathogens [(1)]. Hypercholesterolemia, one of the major risk factors of atherosclerosis, is a major health problem in world [(2,3)], enhances the free radical generation in various ways [(4)]. Prime targets of OFR attack are the polyunsaturated fatty acids in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function [(5)]. Decomposition of peroxidized lipids yields a wide variety of end products. The protective efficiency of antioxidants in hypercholesterolemic patients would depend on the balance between OFR and the availability of antioxidants themselves [(6)]. The

organism's susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defense. Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications [7-9]. Diabetes is usually accompanied by increased production of free radicals [8,10] or impaired antioxidant defenses. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partly known, including activation of transcription factors, advanced glycated end products (AGEs), and protein kinase C [11,12].

The one of major classes of antioxidant enzymes is Glutathione reductase (GR). It is a key enzyme of the antioxidative system that protects cells against free radicals. The enzyme catalyzes the reduction of gutathione (GSSG) to GSH by the NADPH-dependent mechanism. Decreased GSH/GSSG ratio contributes to oxidative stress [13-14]. Due to its important role, this enzyme is more stable than the other cytosolic enzymes and can protect its activity at high temperatures. In the inhibition of GR and disturbance in the cellular prooxidant-antioxidant balance, intracellular GSSG accumulates, and the loss of thiol redox balance may cause loss of cellular homeostasis and numerous diseases [15,16].

Reduced glutathione (GSH) is a major intracellular redox buffer glutathione functions as a direct free-radical scavenger, as a co-substrate for glutathione peroxidase activity, and as a cofactor for many enzymes [17].

The physiological function of Gamma Glutamyl transpeptidase (GGT) enzyme activity as a source of peptide precursors for intracellular GSH re-synthesis, as well as the current clinical concept of its serum activity as the consequence of a compensatory overexpression in response to hepatobiliary dysfunction or alcohol toxic effect, is challenged. The evidence is growing in favor of a detrimental role, Glycated triggering a prooxidant action within the atherosclerotic plaque. Additional investigation would permit the identification among subjects with higher GGT value those with a higher risk of developing clinical disease, allowing definition of the interrelationships with iron metabolism alterations, markers of inflammatory process, of glucose and metabolic disease, and with presence, features, and extent of atherosclerotic vessel disease, to better define the most risky combination for the vulnerable plaque and the best medical strategies for the stabilization of lesions, rather than percutaneous or surgical procedures [18, 19].

Methods

This study included 33 patients with T2DM aged between (30-70) years (19 females and 14 males) and disease duration (1-8 years) with hypercholesterolemia, and 37 patients with hypercholesterolemia alone (23 females and 14 males), who were attending the Diabetic Clinic and Internal Clinic at Al-Kadhymia Teaching Hospital. The patients attended the hospital between (9.00-12.00 am) after an overnight fast. The study also included 54 age-matched normal volunteers (30 female, 23 male) type 1 DM and gestational diabetes were excluded from the study.

Ten milliliters (10 ml) of venous blood were withdrawn from both patients and controls. One milliliter (1 ml) of the blood was added to EDTA tube for HbA1c measurement. The rest of the blood sample was collected in plain tube and centrifuged for 15 minutes at 3000 rpm after being allowed to clot at room temperature for 30 minutes. The separated sera were divided into aliquots and stored frozen at (4 ˚C) for determination of glutathione reductase (GR) and gamma- glutamyl transferease (GGT). Fasting blood glucose, lipid profile, urea and creatinine were done immediately after separation of the serum by the available routine methods. Glycated hemoglobin (HbA1c) was measured by variant HbA1c program which is based on ion-

exchange high performance liquid chromatography (HPLC). Serum GSH concentration was determined by modified method of Elleman, 1959, serum GR by a kit supplied by Randox laboratories Ltd., and serum GGT by a kit from Human Geseli Schaft fur Biochemica and Diagnostica mbH.

Results

From table 1 there are highly significant differences in the mean values of both groups of patients (diabetic hypercholesterolemic DM+HC and hypercholesterolemic HC) and the control group in the following serum biochemical parameters: cholesterol, TG, LDL-c/HDL-c ratio, GSH, GR, GGT (p < 0.001), while a highly significant difference in the mean values of fasting blood glucose between DM+HC patients and both HC patients and control group is seen.

There are highly significant differences in the mean values of GSH and GR between the diabetic hypercholesterolemic patients (DM+HC) and the hypercholesterolemic patients (HC).

Serum cholesterol correlated negatively with both s.GSH and s.GR in the diabetic hypercholesterolemic patients as shown in figures 1 and 2 respectively and negatively with s.GR and positively with s.GGT in the hyper-cholesterolemic patients as shown in figures 3 and 4 respectively. Figures 5 and 6 shows the presence of negative correlations between HbA1c and antioxidants parameters (s.GSH and s.GR) in diabetic hypercholesterolemic patients.

Table 1. Serum parameters involved in the study of hypercholesterolemic patients (HC) and diabetic hypercholesterolemic (DM+HC) patients as (mean±SD)

Group	HC	DM + HC	C
Glucose (mg/dl)	91.75±12.96	177.72±53.51**†	85.30±13.23
Cholesterol (mg/dl)	263.08±23.66†	265.24±16.64	165.20±23.22
TG (mg/dl)	193.78±82.55†	224.33±72.26†	127.05±31.21
LDH/HDL	5.07±1.25†	5.32±1.07†	2.2±0.64
GSH (mmol/L)	0.33±0.031†	0.30 ±0.042*	0.38±0.02
GR (U/L)	60.39 ±6.03†	54.24±5.75**	70±5.2
GGT (IU/L)	56.75 ±11.0†	58.15±8.30†	27.94 ±3.71
HbA1c %	----------	8.25±0.62	----------

GSH: reduced Glutathion, GR: Glutathion reductase, GGT: Y- glutamyl transpeptidase, HbA1c: glycated Hb.

* Significant at p < 0.05 ** significant at p < 0.001 when DM with HC patients compared to HC patients

† significant at p < 0.001 when HC patients and DM with HC patients were compared to their control subjects.

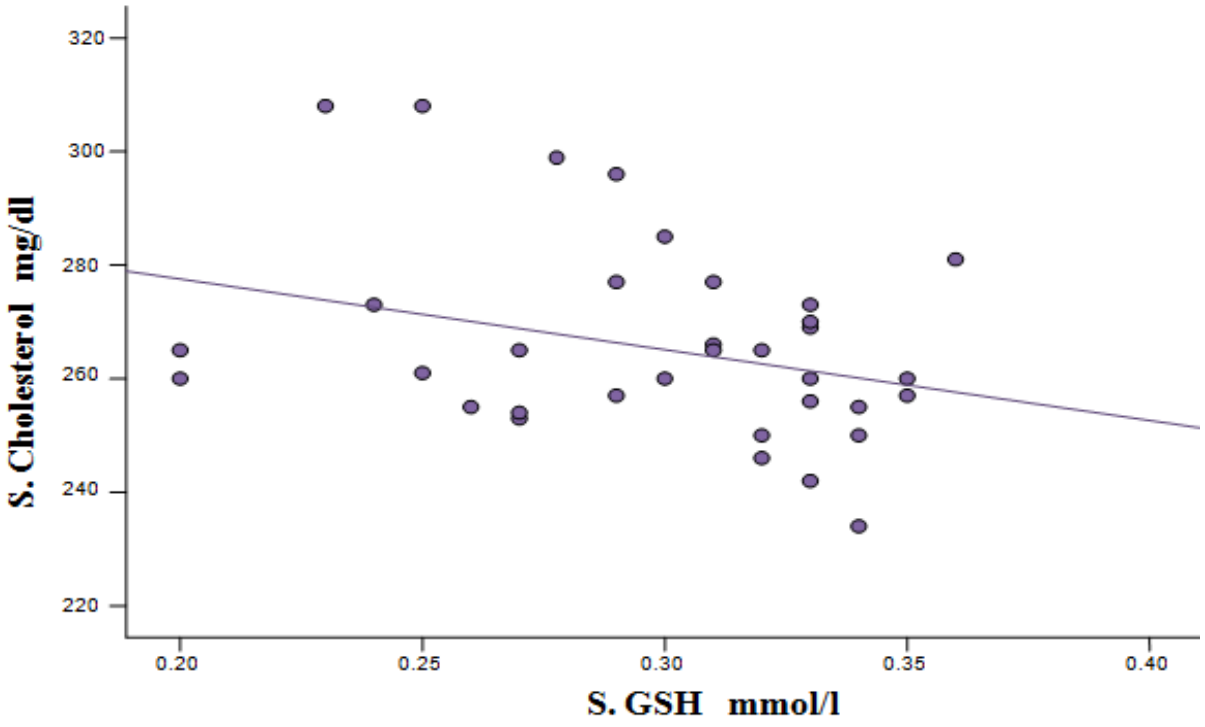

Figure 1. Correlation between serum cholesterol and glutathione (GSH) in diabetic hypercholesterolemic patients (r= -0.34, *p* < 0.05, n= 33).

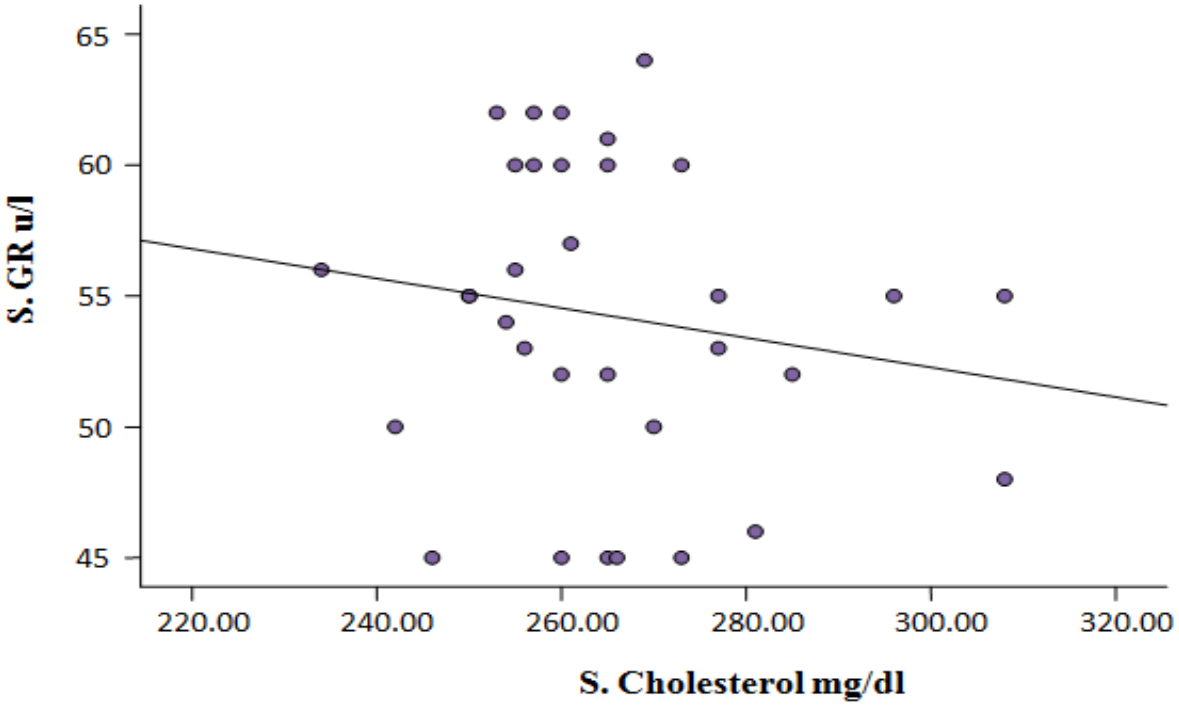

Figure 2. Correlation between S. cholesterol and S. GR in T2DM with hypercholsetermic patients (r= -0.16, *p* < 0.05, n=33)

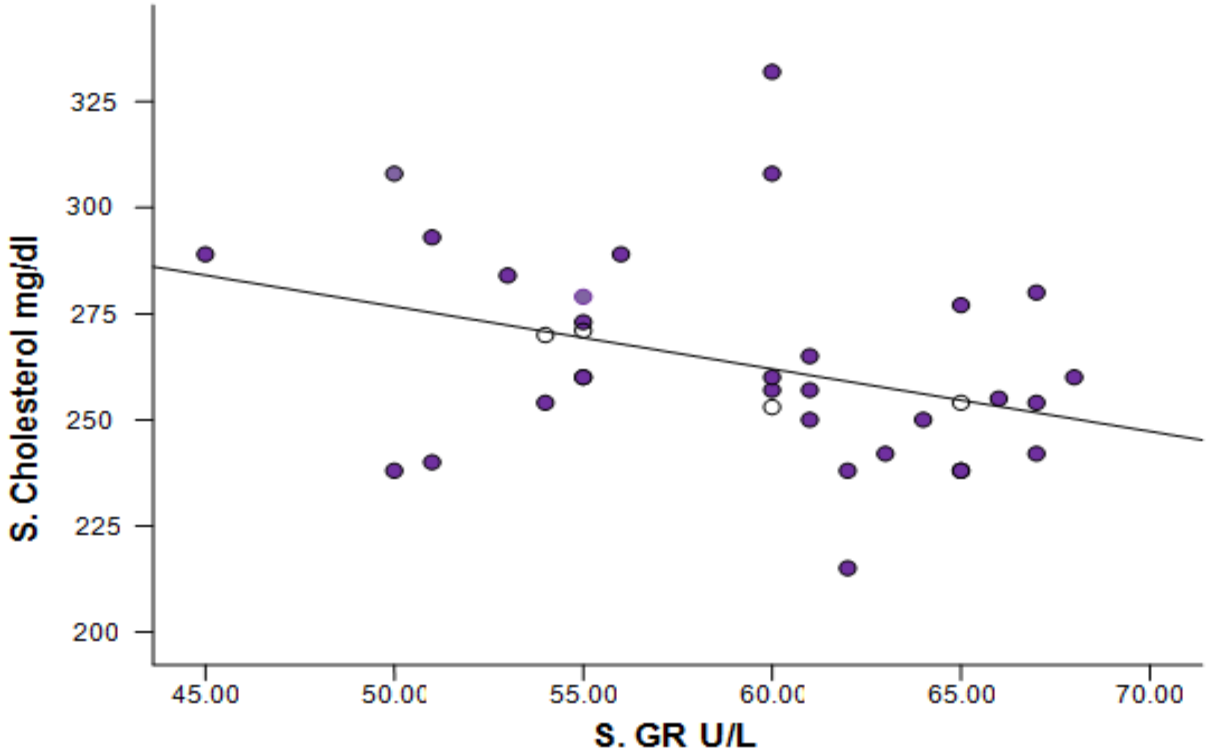

Figure 3. Correlation between S. cholesterol and S. GR in hypercholestrmic patients (r= -0.36, $p < 0.05$, n=37)

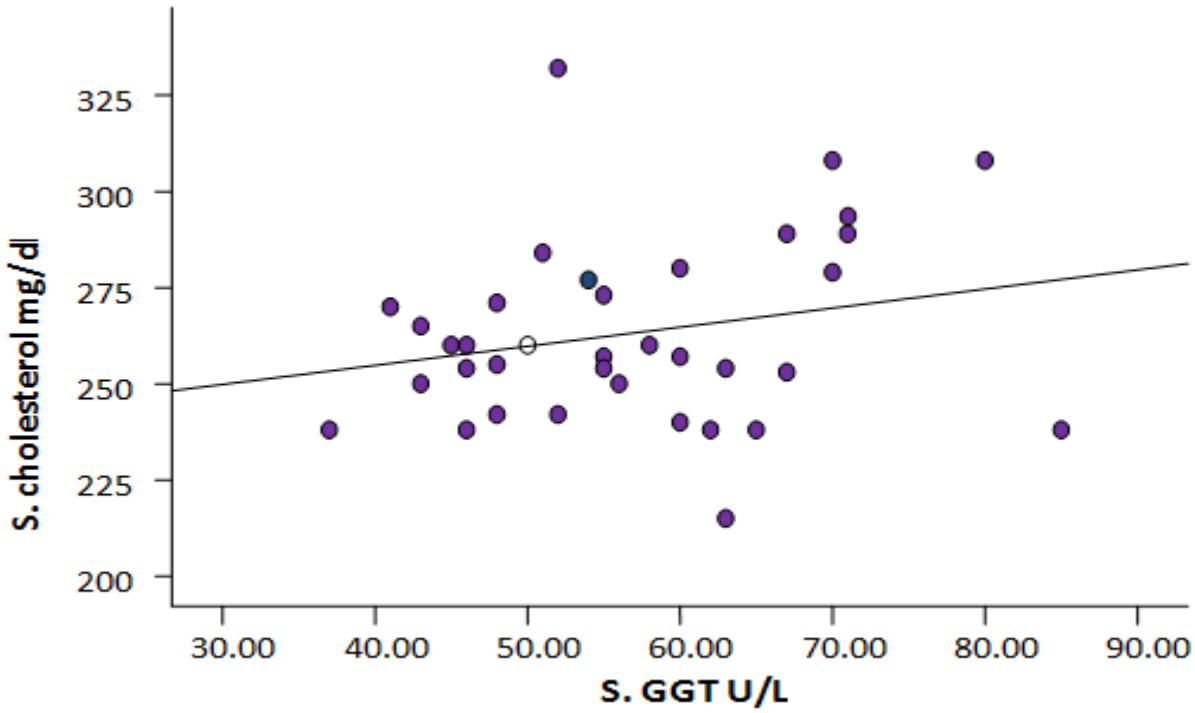

Figure 4. Correlation between S. cholestrol and S. GGT in hypercholsetermic patients (r= 0.23, $p < 0.05$, n= 37)

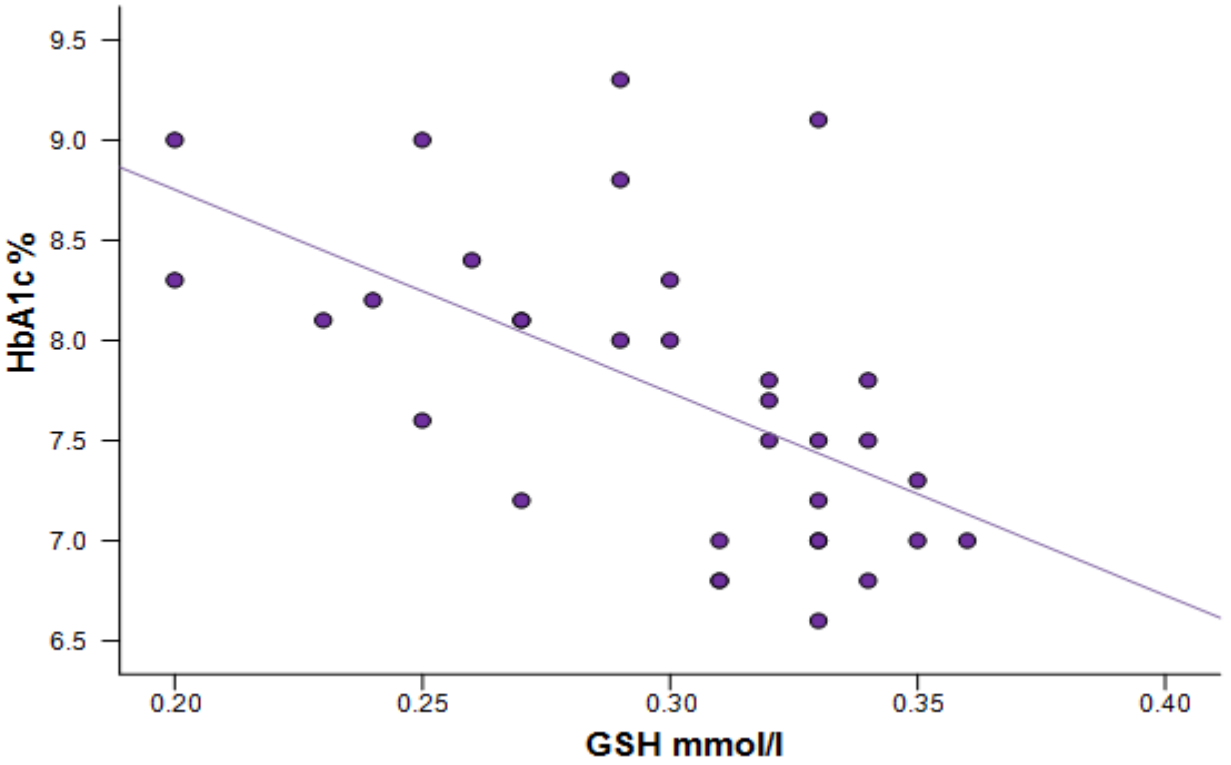

Figure 5. Correlation between serum glutathione (GSH) and HbA1c in diabetic hypercholesterolemic patients (r=-0.35, $p < 0.05$, n=33)

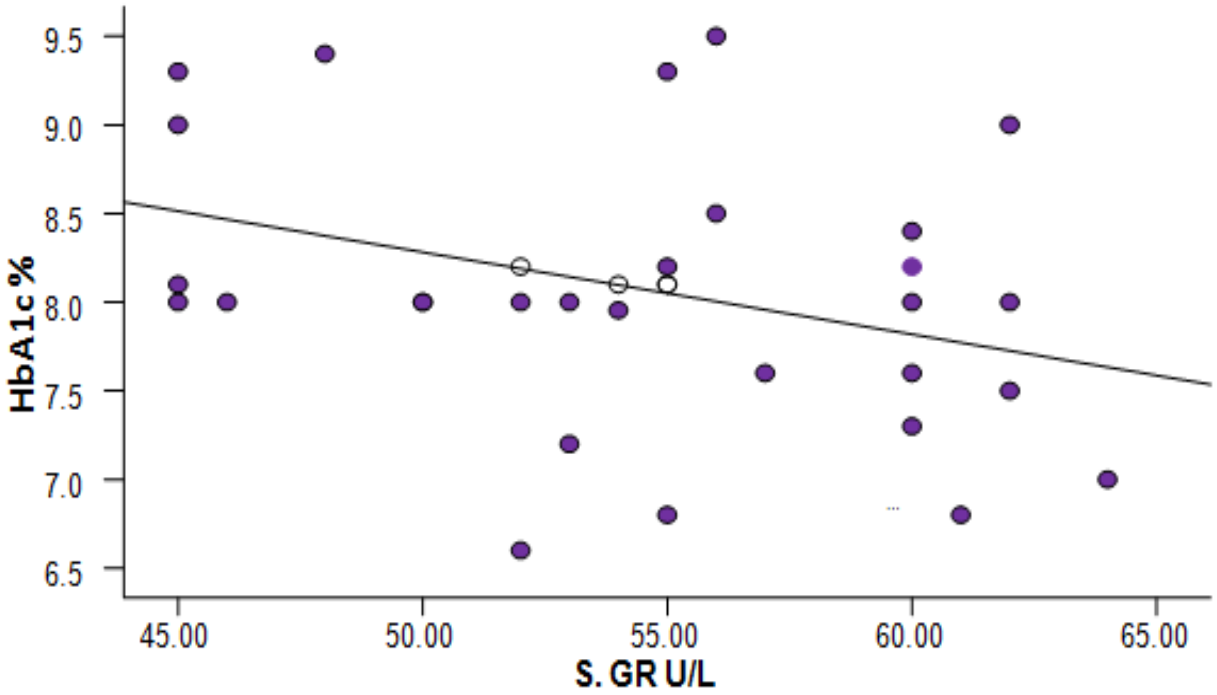

Figure 6. Correlation between serum GR and HbA1c in diabetic hypercholesterolemic patients (r=-0.4, $p < 0.05$, n= 33)

Discussion

The primary findings of this study reveal that there were significant differences in serum glutathione concentration and highly significant differences in glutathione reductase activity between the hypercholsetermic patients and T2DM patients with hypercholestermia. Studies showed high level of oxidative stress in subjects with hypercholesterolemia (risk factors for coronary heart). Researchers at the University of Warwick (UK) have suggested diabetes to be a hidden condition for some patients with coronary heart disease [20]. They found that high levels of oxidative stress in people with coronary heart disease, previously thought to be a maker of the heart condition, could instead indicate a condition of glucose abnormality, such as overt type 2 diabetes [20,21].

The lower GSH, GR and protein with the rise in GGT (as compared to the controls) of the present study reveal decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid profile in these patients (tables 1, 2, and 3).

Sailaja et al in 2003 reported that diabetic humans have shown increased lipid peroxidation and decreased levels of reduced glutathione, glutathione reductase and glutathione peroxidase activities [22].

Glutathione concentration was found to decrease in the liver [23,24]. Kidney, pancreas, plasma, red blood cells, nerve, and precataractous lens of chemically induced diabetic animals [25-28].

Emerging evidence has shown that serum GGT is more than a marker of alcohol consumption. Population-based studies have observed a strong association between serum GGT levels and many cardiovascular disease risk factors [29,30].

After adjusting for alcohol consumption, the factors show a positive association with elevated serum GGT level in the population studies including: old age, male gender, body mass index, smoking, lack of exercise, high blood pressure, heart rate, high blood cholesterol, high blood fasting triglycerides, high blood LDL cholesterol, low blood HDL cholesterol, high fasting glucose, and, among women, menopause and use of oral contraceptive [31]. These relationships have been shown to be strong even within the normal range of GGT levels. In addition, several prospective studies have shown that baseline serum GGT level is an independent risk factor for the development of heart disease, hypertension, stroke, and type 2diabetes, regardless of alcohol consumption [29,30]. Experimental findings that cellular GGT changes its role from an antioxidant to a pro-oxidant in the presence of a transition metal such as iron are of interest because iron can increase cellular GGT during oxidative stress [30]. The prospective study done by Ruttman and colleagues in 2005 on 163 944 Austrian adults studied for 17 years showed that GGT was independently associated with cardiovascular mortality. This was found to be true in both sexes, with a clear dose-response relationship, and with a stronger prognostic significance of GGT in younger participants [33]. Prospective cohort studies have revealed that plasma GGT activity exhibits a positive association with coronary artery disease [34].

Another three-year follow up study has shown that GGT increases in type 2 diabetes in middle aged men and women [35].

Increased oxidative stress as measured by markers of oxidative stress has been shown to be increased in type 2 diabetes mellitus. Despite strong experimental evidence indicating that oxidative stress may determine the onset and progression of late-diabetes complications, controversy still exists about whether the increased oxidative stress is merely associative rather than causal in DM [36,37] .

The negative correlation between serum cholesterol and each of serum GSH and GR in both groups of patients (Figures 1 and 3) may confirm the fact that both hypercholesterolemia and diabetic hyper-cholesterolemia will lead to a

decrease in the levels of antioxidant enzymes and an increase in the levels of free radicals , being more obvious in the later [36].

Alteration in antioxidant defenses in diabetes might lead to the development of diabetic induced complications. Evidence has accumulated indicating that oxidative stress may play an important role in the etiology of diabetic complications. The poor glycemic control in diabetic patients was associated with decreased free radical scavenging activity, something confirmed by the presence of a negative correlation between the levels of HbA1c and each of GSH and GR of the present study (Figures 5 and 6). In hyperglycemia, glucose undergoes auto-oxidation and produces free radicals that in turn lead to peroxidation of lipids in lipoproteins. Elevated levels of lipid peroxidation, hydroperoxide and conjugated diene seen in diabetic patients are clear manifestations of excessive formation of free radicals resulting in tissue damage [37,38], and this may lead to the speculation that elevated oxidative stress in coronary heart disease is not a marker for the heart condition only, but may indicate a condition of glucose abnormality similar to that of type 2 diabetes mellitus.

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Correspondence to: Zainab A.A.Al-Shamma
E-mail: z.alshamma@gmail.com
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