PhD Thesis Summary

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"Effect of Glycemic Control on Soluble RAGE and Oxidative Stress in Type 2 Diabetic Patients"

The present study was designed to evaluate the effect of glycemic control on soluble receptor of advanced glycation end product (sRAGE) and some oxidative stress markers in type 2 diabetic patients.

This study was conducted in the Out-patient Clinic of Beni-Suef University Hospital between September 2011 and June 2012 and comprising 90 subjects who were subdivided into 20 healthy control volunteers and 70 patients with type 2 diabetes mellitus (T2DM).

All patients enrolled in the study fulfilled the following criteria: age between 35-70 years; receiving stable antidiabetic therapy (sulfonylurea, metformin, thiazoldinedione and/or insulin) for at least 6-8 months and no history of ketoacidosis. In hypertensive diabetic patients submitted to the study, the antihypertensive treatments were angiotensin converting enzyme inhibitors, angiotensin receptor blockers, Ca^{2+} channel blockers, β-blockers and/or diuretics for at least 6-8 months.

Exclusion criteria included the following: clinically significant hepatic, neurological, endocrinologic or other major systemic diseases, such as malignancy; elevated plasma transaminase activities over twice the upper limit of normal; elevated plasma creatinine concentrations (>150 μmol/L or 1.7 mg/dl); acute major cardiovascular events in the previous 6 months; acute illnesses; current evidence of acute or chronic inflammatory diseases and hormone replacement therapy for women subjected to the study. Exclusion criteria also included treatment with glucocorticoids, antineoplastic agents, psychoactive agents, bronchodilators, statins or vitamin supplements.

The patients enrolled in the present study were classified according to glycemic control [*good glycemic control* (glycated hemoglobin (HbA_{1c}) ≤ 7.0 %) and *poor glycemic control* (HbA_{1c} > 7.0 %)] with or without hypertension (SBP > 140 mmHg or DBP > 90 mmHg) along with normal subjects as follows:

I- Control healthy subjects [Group (1)]: It included twenty healthy individuals, age– and body mass index (BMI)–matched with diabetic patients.

II- Good controlled diabetics [Group (2)]: It was composed of twenty eight good controlled diabetics (GCD).

III- Poorly controlled diabetics [Group (3)]: It was composed of forty two poorly controlled diabetics (PCD).

Ten milliliters of venous blood samples were withdrawn after 12-14 hours overnight fast from each subject enrolled in the study. Each blood sample was collected into tubes containing EDTA and divided into 2 aliquots. The first aliquot was of 4 ml whole blood used for estimation of glutathione (GSH), HbA_{1c} and superoxide dismutase (SOD) activity. The second aliquot was of 6 ml blood centrifuged at 2000 x g for 10 minutes to obtain plasma for estimation of plasma glucose levels, alanine transaminase (ALT) and aspartate transaminase (AST) activities, plasma creatinine and lipid profile. The remaining plasma was stored at –20ºC for subsequent estimation of C-peptide, sRAGE, vascular cell adhesion molecule-1(VCAM-1), oxidized low density lipoprotein (ox-LDL) and total nitric oxide (NO_x) levels.

The present study showed a significant increase in fasting plasma glucose (FPG) levels in both diabetic groups compared with normal controls and increased HbA_{1c} levels in PCD compared with GCD. The increase in HbA_{1c} is attributable to the intracellular hyperglycemia which increases non-enzymatic attachment of glucose molecules to primary amino groups of hemoglobin protein, forming stable Amadori products

such as the HbA_{1c} adduct. Additionally, a significant positive correlation was found between HbA_{1c} and FPG levels in all diabetic patients in the current study. The lower FPG and HbA_{1c} levels in GCD compared with PCD may be attributed to intensive glycemic control with different hypoglycemic drugs such as sulfonylureas and metformin.

The current work showed a significant decrease in sRAGE levels in PCD compared with normal control. Moreover, no significant difference in the mean plasma sRAGE levels was observed when comparing GCD with either PCD or with normal control. The sRAGE level in PCD may be reduced due to excessive binding to circulating AGEs ligands. Increased ligand burden may consume all existing sRAGE and/or endogenous mechanisms that release sRAGE may be impaired.

The plasma VCAM-1 levels showed a significant increase in PCD compared with normal control. Moreover, no significant difference in the mean plasma VCAM-1 levels was observed when comparing GCD with either PCD or with normal control. Poor glycemic control and increased glucose levels may be responsible for the significantly higher levels of VCAM-1 in PCD. Hyperglycemia leads to increased production of AGE which stimulates vascular inflammation and VCAM-1 expression. This may explain the significant elevation of VCAM-1 in PCD but not in GCD. The sRAGE, which is important in the capture of AGE and prevents the effect of AGE on signaling and alteration of cellular properties, was significantly decreased in PCD but not in GCD. This may emphasize the hypothesis that increased AGE levels in PCD cause elevation of VCAM-1. It is worth mentioning here that an inverse correlation between sRAGE and VCAM-1 was found in the present study.

The current study revealed that there was no significant difference in plasma NO_x levels in GCD and PCD compared with either normal control or with each other. No change in SOD activities was found in diabetic

groups compared with normal controls which may protect $NO^{\bullet}$ from the deleterious $O_2^{\bullet-}$ and so, NO_x level in diabetic patients didn't differ from that of normal subjects.

The present work revealed that there was no significant difference in plasma ox-LDL levels in GCD and PCD compared with either normal control or with each other. Concomitant elevation of circulating glucose and ox-LDL levels is not usually found in well-controlled DM patients, but can be seen in uncontrolled T2DM patients or in some stress conditions, such as in inflammation or infection and during hospitalization. Thus, the exclusion of conditions of inflammation or infection in patients of the present study may explain the unchanged ox-LDL levels. Another explanation for the unchanged ox-LDL levels in diabetic patients in the present study may be the treatment with sulfonylurea, insulin and/or metformin which have antioxidant effects.

The present results showed that there was no significant difference in blood SOD activity in GCD and PCD compared with either normal control or with each other. It is possible that changes in SOD activity may occur in early stages of diabetes. Patients in the present study had had diabetes for a long time and had been on long-standing hypoglycemic agents which may be a possible explanation for the unchanged SOD activity in these patients.

The current study revealed that there was a significant decrease in blood GSH levels in GCD and PCD compared with normal control. Decreased GSH levels in patients with diabetes may be caused by different pathways including: increased activity of sorbitol pathway which depletes NADPH, and so limits the reduction of GSSG to GSH; decreased activity of glucose-6-phosphate dehydrogenase in hexose monophosphate shunt in DM which generates NADPH; and *finally* passage of GSSG *via* erythrocyte membrane which inhibits its reduction

to GSH. Another cause for the lower GSH levels in diabetes may be the decreased amino acids necessary for GSH synthesis: L-cysteine, L-glutamate and L-glycine.

From this study we can conclude that improvement of glycemic control increases sRAGE and decreases VCAM-1 levels, thus inhibiting the deleterious cascade that results from activation of AGE/RAGE axis. Also, the use of certain hypoglycemic drugs with antioxidant properties in treatment of type 2 diabetes may provide benefit *via* reduction of oxidative stress.

Further studies are needed to investigate whether sRAGE levels are a reproducible and predictive biomarker for the development of diabetic vascular complications. Also, further investigations are needed for the development of strategies aiming at raising sRAGE levels.

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