Eryptosis and oxidative damage in type 2 diabetic mellitus sedentary workers with chronic kidney disease.

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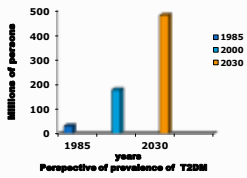
Diabetes Mellitus Type 2 (T2DM2)

It is metabolic disease characterized by disturbances in carbohydrate, lipid, and protein metabolism.
Type 2 diabetes mellitus (T2DM) is associated with reduced insulin sensitivity on its target tissues (insulin resistance)

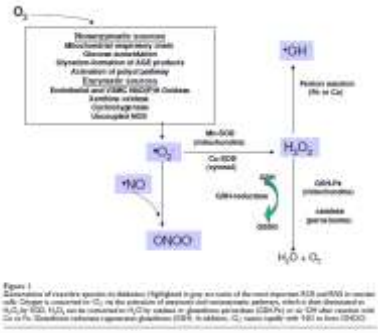
Diabetes mellitus (DM) is the most prevalent metabolic disease and represents a serious clinical and public health problem.
The International Diabetes Federation estimates that 285 million people around the world have diabetes.
This total is expected to rise to 438 million within 20 years.
Each year another seven million people develop diabetes
T2DM occurs predominantly in adults over 30 years of age.



*Federación Mexicana de Diabetes A.C., *(IDF, Atlas de Diabetes. Cuarta Edición), Secretaria de Gobernación, http://www.dof.gob.mx/documentos/3868/Salud/Salud.htm IDF. (2009) Diabetes Atlas at the 20th World Diabetes Congress in October 2009, Montreal, Canada. http://www.diabetesatlas.org/. Golbidi S, et al. (2011) Curr Diabetes Rev 7(2):106–125*
3. American Diabetes Association (2007) Diagnosis and classification of diabetes mellitus. Diabetes Care, http://care.diabetesjournals.org/content/30/suppl_1/S42.full. Accessed February, 2011 Kaneto H, et. al Mediat Inflamm 2010:453892

Under diabetic conditions, chronic hyperglycemia may induce an increase of reactive oxygen species and nitrogen species (ROS/RNS).
Oxidative insult deteriorates pancreatic b-cell function and amplifies insulin resistance which leads to the aggravation of T2DM.

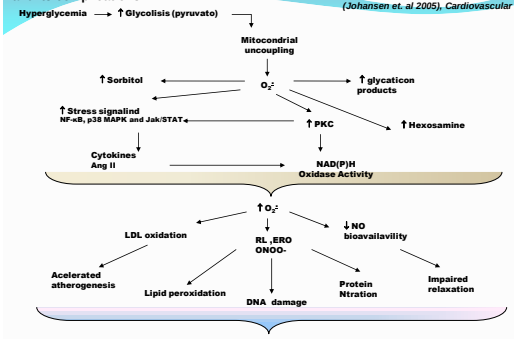
In diabetes, the loss of balance in the pro-oxidant/antioxidant equilibrium can damage cellular macromolecules, leading to DNA and protein modification and lipid peroxidation



(Johansen et. al 2005), Cardiovascular Diabetology

Oxidative stress has been shown to be partly responsible for the progression of diabetes and its complications

(Johansen et. al 2005), Cardiovascular Diabetology



Diabetes-induced oxidative damage may be more important in erythrocytes compared to other cells, because of their high content of iron and polyunsaturated fatty acids, and their role as oxygen transporters, with a high exposition to free radicals

Suicidal death of erythrocytes (eryptosis) is characterized by cell shrinkage, membrane blebbing, activation of proteases and phosphatidylserine exposure at the outer membrane leaflet.

Eryptosis is triggered by erythrocyte injury following several stressors including osmotic shock, oxidative stress, ligation of cell membrane antigens and energy depletion

Phosphatidylserine at the erythrocyte surface is recognized by macrophages which engulf and degrade the affected cells

Institute of Physiology Tübingen
Lang, K.S.et al. (2005). Cell Physiol Biochem. Calderón Salinas y col. REB. .25. 2006.

Eryptosis and Diabetes Mellitus

Eryptosis may be a mechanism of defective erythrocytes to escape hemolysis. Excessive eryptosis favours the development of anemia.

Conditions with excessive eryptosis include iron deficiency, lead or mercury intoxication, sickle cell anemia, thalassemia, and glucose-6- phosphate dehydrogenase deficiency, malaria and infection with hemolysin-forming pathogens. Recent study showed that hyperglycemia in erythrocytes of type 1 DM patients increases the percentage of circulating erythrocytes exposing phosphatidylserine (PS) at the cell surface.

Exposure to methylglyoxal, a reactive dicarbonyl compound that is formed as a metabolic by product of glycolysis. Enhanced PS exposure of circulating erythrocytes and eventually resulting in anemia and microcirculatory disequilibrium.

Elevated PS exposure in erythrocytes might be a risk factor for anemia and contribute to its development in patients with CRF, treated with peritoneal dialysis.

PS exposure is not limited to erythrocytes alone; this condition can also be acquired by other cells

Lang KS, et al. Mechanisms of suicidal erythrocyte death. Cell Physiol Biochem 15(5):195–202- Nicolay JP, et al (2006) Cell Physiol Biochem 18(4–5):223–232 . Lang PA, et al., Cell Death Differ 12:415–428m Lang F, et. al Cell Physiol Biochem

Materials and methods

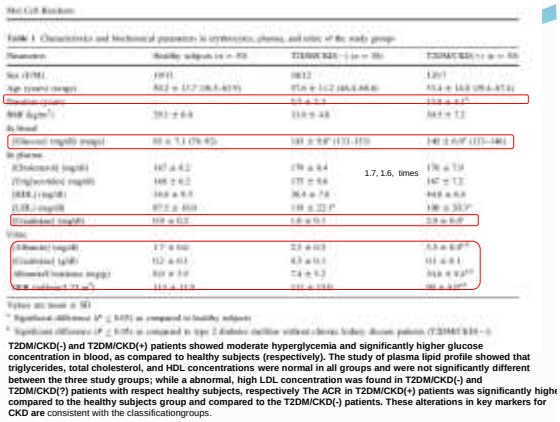
The study was designed to determine the presence of eryptosis in T2DM patients with or without CKD (T2DM/CKD(+) and T2DM/CKD(-)). In addition to this, we measured plasma total antioxidant capacity as well as oxidative damage, GSH/GSSG ratio of all groups and its possible association with the PS externalization in erythrocytes.

This study included 90 sedentary individuals (Governmental workers): 30 T2DM patients without CKD (T2DM/CKD(-)); 30 T2DM patients with CKD (T2DM/CKD(+)); and 30 health volunteers. T2DM patients were selected and characterized according to the diagnostic criteria recommended by the Expert Committee on the Diagnosis and Criteria for the diagnosis of diabetes: Fasting plasma glucose during an oral glucose tolerance test (OGTT). World Health Organization.

Chronic kidney disease (CKD) was defined by the persistent presence (3>months) of kidney damage. In accordance with the National Kidney Foundation, kidney damage was diagnosed by the measurement of albumin to creatinine ratio (ACR) in a random spot urine collection for detection of microalbuminuria. Glomerular filtration rate (GFR) was used to assign a stage of CKD. This group included only stage 1 patients of CDK (kidney damage with GFR C 90 ml/min/1.73 m2, K/DOQI (Clinical Practice Guidelines for Chronic Kidney Disease:

The patients in the study had glycated hemoglobin (HbA1c) within the normal range (>7%) for the past 3 years. The patients were controlled with diabetic standard medications [metformin (1,700 mg/day) and glibenclamide (5 mg/day)] diets and exercise.

Patients were excluded if they had: symptoms of hyperglycemia or acute hyperglycemic crisis, a random plasma glucose 200 mg/dland use of antioxidants or other medicaments in 6 months previous to our study. This study was accepted by Local Research Ethics and Investigation Committee of Mexican Social Security Institute, Durango, Dgo. (ID: R-2007-901-5). Subjects came from the same geographical area, and, in accordance with the Helsinki Declaration, a voluntary written consent was obtained from each participant.

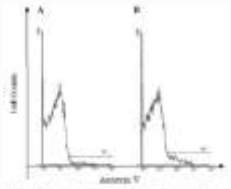


1.7, 1.6, times

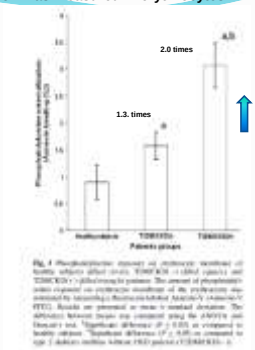
T2DM/CKD(-) and T2DM/CKD(+) patients showed moderate hyperglycemia and significantly higher glucose concentration in blood, as compared to healthy subjects (respectively). The study of plasma lipid profile showed that triglycerides, total cholesterol, and HDL concentrations were normal in all groups and were not significantly different between the three study groups; while a abnormal, high LDL concentration was found in T2DM/CKD(-) and T2DM/CKD(?) patients with respect healthy subjects, respectively The ACR in T2DM/CKD(+) patients was significantly higher compared to the healthy subjects group and compared to the T2DM/CKD(-) patients. These alterations in key markers for CKD are consistent with the classificationgroups.

To evaluate the eryptosis, PS externalization was measured in erythrocytes:

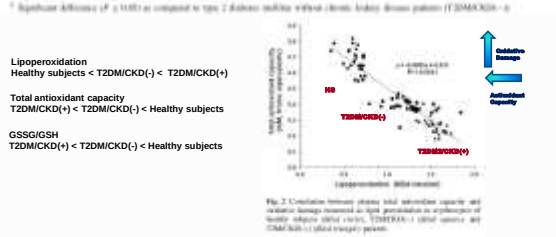
Externalization of PS in erythrocytes of healty subject and T2DM (-) patient



% PS Externalization
HS < T2DM/CKD(-) < T2DM/CKD(+)



The biomarkers of oxidative damage to lipids in erythrocytes were elevated in diabetic patients.

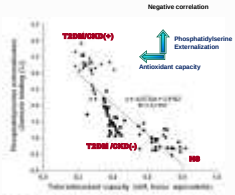


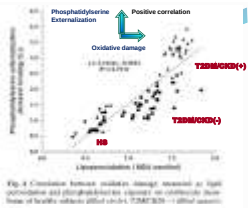
Lipoperoxidation
Healthy subjects < T2DM/CKD(-) < T2DM/CKD(+)

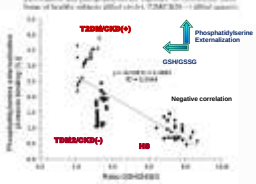
Total antioxidant capacity
T2DM/CKD(+) < T2DM/CKD(-) < Healthy subjects

GSSG/GSH
T2DM/CKD(+) < T2DM/CKD(-) < Healthy subjects

These results suggest that the increase in PS externalization may relate with both an increase in oxidative damage and a decrease antioxidant capacity.







Conclusions

Oxidative damage of lipids erythrocytes were increased in diabetic patients. The highest lipoperoxidation was found in T2DM/CKD(+).

The lower plasma total antioxidant capacity, GSH/GSSG ratio, and GSH in erythrocytes were found in T2DM/CKD(+) patients.

A negative correlation was found between plasma total antioxidant capacity and oxidative damage.

Phosphatidylserine (PS) externalization was measured in erythrocytes to evaluate eryptosis.

Annexin binding in erythrocytes of T2DM/CKD(+) patients was higher than in healthy subjects and T2DM/CKD(-) patients.

A positive correlation between lipoperoxidation and PS externalization in erythrocytes was found. This work showed that the erythrocytes of diabetic patients have increased oxidative damage, a reduction of antioxidant systems and more erythrocyte PS externalization.

The duration of diabetes and the presence of CKD increase both oxidative damage and eryptosis. It is possible that a longer time of evolution induces an increase in erythrocyte oxidative damage and the consumption of blood antioxidant systems, adding to the osmotic stress in CKD and so contributes to an increase in PS externalization in diabetic patients.