

Oxidative status for Iraqi young female patients with diabetes mellitus type one

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Abstract:

The study attempts to investigate the relationship between diabetes patients and controls represented by levels of (fasting blood sugar, glutathione S transferase, blood urea, and Malondialdehyde) in Iraqi young female patients with DM1 without complications. The study groups consisted of 66 patients with DM1 (15-30 years) and 22 controls young female. It was found that the GST was higher in diabetes patients compared with controls but less over time where (group one slightly higher than group two and more higher than group three) and were found GST not significantly in diabetes patients compared with controls. In the MDA it was found that the group one and group two of diabetes patients were significantly (p value < 0.05) between diabetes patients and controls and found MDA less over the time.

I. Introduction

Diabetes mellitus is a clinically and genetically heterogeneous group of metabolic disorders manifested by high levels of glucose in the blood. The hyperglycemia is the result of deficiency of insulin secretion caused by pancreatic B-cell resistance to the action of insulin in liver and muscle or combination of these, frequently this metabolic disarrangement is associated with alteration in adipocyte metabolism, diabetes is a syndrome and it is now recognized that chronic hyperglycemia leads to long term damage to different organs including the heart, eyes, kidneys, nerves and vascular system. There are several etiologies for diabetes and although establishing the type of diabetes for each patient is important, understanding the pathophysiology of the various forms of the disease is the key appropriate treatment, the current classification of diabetes is based upon the pathophysiology of each form of the disease.

Type one diabetes (B-cell destruction usually leading to absolute insulin deficiency) this form of diabetes, which accounts for only 5-10% of those with diabetes, previously encompassed by the terms insulin dependent diabetes type one diabetes result from a cellular mediated auto immune destruction of the B-cell of the pancreas, some forms of type one diabetes have no known etiologies.

Autoimmune destruction of B-cells has multiple genetic predisposition and is also related to environmental factors that are still poorly defined, although patients are rarely obese when they present with this type one diabetes, the presence of obesity is not incompatible with the diagnosis. Increasing evidence in both experimental and clinical studies suggests that oxidative stress plays a major role in the pathogenesis of the both types of diabetes mellitus, free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes, increase lipid peroxidation and development of insulin resistance, these consequences oxidative stress can promote the development of complications of diabetes mellitus, change in oxidative stress biomarkers, including malondialdehyde, glutathione transferase, blood urea. Glutathione transferase have historically also been called glutathione S transferase, and it is the latter name that gives rise to the widely used abbreviation GST, these enzymes catalyze nucleophilic attack by reduced glutathione (GSH) on non polar compounds that contain an electrophilic carbon, nitrogen or sulphur atom.

GST is a super family of enzymes that catalyzes the conjugation of xenobiotics and endogenous substances with glutathione, and thereby play a significant role in the inactivation and occasionally the activation of many drugs and xenobiotics, a number of potentially toxic electrophilic xenobiotics (such as certain carcinogens) are conjugated to the nucleophilic.

MDA is the most marker that is used to investigate the presence of lipid peroxidation in biological system, the pathways that lead to marker from lipid peroxidation, MDA is a significant by product during enzymatic synthesis of prostaglandin, it can be removed by renal clearance, it plays a significant role in DNA damage, sister chromatid exchange and carcinogenesis, MDA is a three carbon molecule with two aldehyde groups which renders it highly reactive with other biomolecules, MDA circulates in plasma either bound to protein or in free form, another product of MDA is generated in vitro from decomposition of lipid hydroperoxides during sample preparation.

Urea is the chief end products of protein metabolism in the body the importance of the urea concentration in the blood lies in its value as an indicator of kidney function, Azotemia(an abnormal increase in plasma urea level) ,they are two types of azotemia the first prerenal azotemia is caused by impaired perfusion of the kidneys due to decreased cardiac output or for any of the former causes, the second post renal azotemia,is caused by an obstruction in the urine out flow such as nephrolithiasis,prostatism,and tumors of the genitourinary tract, the clinical significance of the urea level is usually determined in conjugation with theplasma creatinine level, in prerenal azotemia an increase in the plasma urea level is usually associated with anormal creatinin level where as postrenal azotemia there is an increase inboth the urea and plasma creatinine levels decrease in the urea plasma level may be associated with acute dehydration, malnutrition, pregnancy.

Materials and Methods

Patient and design. The study group consisted of 66 young female (15-30)years with DM1 divided to three groups(5,10,15)years according to duration of disease and they had no complication , the number of the groups(24,22,20)respectively, this samples collected from the center of diabetes /almustansria university, in January, February 2013.

Materials.trichloroaceticacide(BDH,England),thio barbutaricacid(H&w,Germany),deionization distilled water.

Chloro di nitro benzene (H&W,Germany) phosphate buffer slain (Biomaghreb,sa,france),reduced glutathione(BDH,England), Enzyme reagent ureas>500 U/mL,bufferd chromagon(phosphate buffer20mmol/l PH6.9,EDTA2mmol/l,sodium salicylate 60mmol/l sodium nitroprusside3.4mmol/l) alkaline hypochlorite,sodium hypochlorite 10mmol/l,NAOH150mmol/l,Urea standard urea50mg/dl,(spinreact,spain).

Determination of Urea in serum. Urea determined by bring reagents and samples to room temperature, then pipetted in to acuvette 1.0 ml of working reagent in the blank ,sample,standerd

Tubes and pippete10 μ l from sample in the sample tube and 10 μ l in the standard tube, mixed and incubated for 5minutes at 37c,then pipetted 1.0ml from reagent to the blank ,sample, and standard tubes, mix thoroughly and incubated the tubes for 5 minutes at 37c,read the absorbance(A) of the samples and the standard at 600nm against the reagent blank.

Determination of Glutathione -s- transferase. Was determined by placed 900 μ L of enzyme cocktail (980 μ L PBS PH6.5,10 μ L of 100mM CDNB ,10mM glutathione) into 1.5 ml quartzes cuvette , incubated at 30c in spectrophotometer for 5min , to the blank cuvete added100ml PBS and zero spectro, 100ml were added to sample cuvette and mixed, measured absorbance at 340nm for five-minute.

Determination of fast blood Glucose. Was determined by clinically method in the early morning by take drop of blood from patient with diabetes type one were the patient fasting measured the glucose by the diabetes apparatus.

Determination of Malone di aldehyde.Was determined by placed 150 μ L of sample(serum),1ml of TCA(17.5%),1ml of TBA(0.6%) in the tube and placed 1ml of TCA(17.5%) ,1ml TBA(0.6%) in another tube (blank),all tubes mixed by vortex and incubated in100c for 15 minute,and all tubes were cooled ,then placed 1ml TCA(70%) in the sample tube and 1ml TCA(70%) in the blank tube mixed and staled for 20 minute at room temperature, then centrifuged 4000 rpm for 15 minute and the supernatant was taken out for mesearing at 532 nm.

Results and Discussion

Glutathione s-transferase constitute a major protective mechanism against oxidative stress low levels of GSH in diabetes patients are found to potentiate the effects of the increased reactive oxygen species , increased of GST activity in diabetes patients independent of their glycemic status there is no significant difference between diabetics and non diabetics.

Increase total GST levels may be due to a compensatory mechanism of anti oxidant to combat the oxidative stress in diabetic conditions with or without complication. The regulation of GST

subjected to a complex set of endogenous and exogenous parameters , as well as large number of xenobiotics inducing agents.

The chronicity of the disease along with treatment modalities might have played a significant role in the subjects included in diabetic group were enrolled without considering their glycemic control status.

Urea in the serum was found not significantly in three groups compared with control groups that mean no defect in kidney functions because all patients groups had diabetic without complications.

The increase of MDA levels in group one and group two reflected to an increase in lipid peroxidation initiated by ROSs, so that imbalance between anti oxidant production and removal of ROSs lead to oxidative stress, but in group three MDA levels decreased and were found not significantly compared with controls because different reasons: no system in the life style, lack of sport, no right nutrition, long time of insulin doses, commitment of periodic examination, genetics, shocks, and another diseases.

The study attempts to investigate the relationship between diabetes patients and controls represented by levels of (fasting blood sugar, glutathione_s transferase, blood urea, and Malone di aldehyde) in Iraqi young female patients with DM1 without complications. The study groups consisted of 66 patients with DM1 (15_30 years) and 22 controls young female. It was found that the GST was higher in diabetes patients were compared with controls but less over time where (group one slightly higher than group two and more higher than group three) and were found GST not significantly in diabetes patients were compared with controls. In the MDA It was found that the group one and group two of diabetes patients were significantly (p value < 0.05) between diabetes patients and controls and found MDA less over the time.

Table 1: GST activity in all patient groups and control group

Groups	Controls	Group1	Group2	Group3
N	22	24	22	20
Mean	216.6127	255.5188	254.5486	187.4253
Std. Dev.	65.5468	98.7162	72.9880	93.9360
Std. Error	13.9746	20.1503	15.5611	21.0047
probability		0.028	0.725	0.699

The results are presented as GST activity in group one significantly but in group two and three not found any (significantly relation between diabetic patients and control groups where p value < 0.05)

Table 2: MDA concentration in all patient groups and control group

Groups	Controls	Group1	Group2	Group3
N	22	24	22	20
Mean	1.2020	3.3573	2.6356	1.3323
Std. Dev.	1.1752	3.2152	2.0889	1.1932
Std. Error	0.2932	0.7175	0.6305	0.4457
probability		0.001	0.0001	0.147

The results are presented as MDA concentration in group one and group two are significantly but in group three not found any significant relation between patient and controls where p value is < 0.05

Table 3: urea in serum concentration in all patient groups and control group

Groups	Controls	Group1	Group2	Group3
N	22	24	22	20
Mean	23.5455	25.3750	25.5909	23.6500
Std. Dev.	3.3198	4.6794	4.9728	3.5433
Std. Error	0.7078	0.9551	1.0602	0.7923
probability		0.145	0.111	0.936

The results are presented as urea in serum in group one, two and three not found any significant relation between patient groups and control group. P value < 0.05 .

Table 4: fast blood glucose concentration between all patient groups and control group

Groups	Controls	Group1	Group2	Group3
N	22	24	22	20
Mean	103.4091	265.05417	242.0455	243.9500
Std. Dev.	3.2755	87.77490	109.0777	85.6292
Std. Error	0.6983	17.91698	23.2554	19.1472
probability		0.0001	0.0001	0.0001

The results presented as fast blood sugar in group one, two and three we are found group one ,two and group three are significantly relation between diabetic patient and control p value(<0.05).

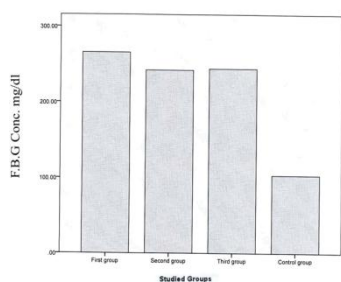


Fig.1: fasting blood glucose concentration between diabetic patients and control

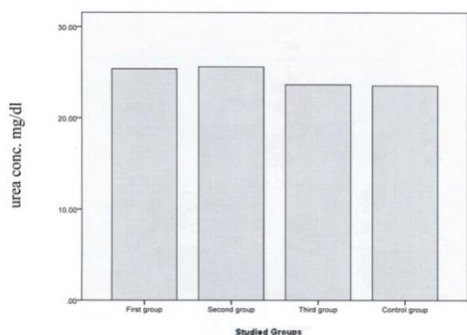


Fig. 2: urea concentration in serum between diabetic patients and control

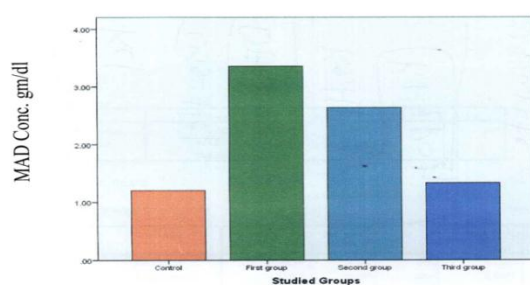


Fig. 3: MDA concentration between diabetic patients and control.

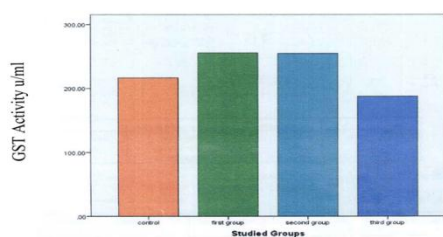


Fig. 4 : GST activity between diabetic patients and control

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