Effects of Sulfonylureas Treatment on Apoptosis, Total Superoxide Dismutase activity and Some Biochemical Parameters in Type 2 Diabetic Patients

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Abstract

The purpose of this analysis was to evaluate the the influence of anti-diabetic drugs (sulfonylureas) in type two diabetic patients and the risk of developing diabetes complications related to kidney disease (Diabetic Nephropathy **)** compared to healthy subjects by measuring influence of some apoptosis factors (serum Tumor necrosis factor soluble Receptor-1(TNF-R1) and TNF-related apoptosis-inducing ligand receptor-2 (TRAIL-R2)) concentrations , Serum Small mothers against decapentaplegic (Smad1) as anew marker of diabetic nephropathy (DN) ,Superoxide dismutase concentration (SOD) **,**Glycated hemoglobin (HbA1c%) , Fasting plasma glucose (FPG) ,insulin concentration , insulin resistance (IR) ,urea and creatinine .A samples of convenience composed of 17 type 2 diabetic patients with sulfonylureas monotherapy and 17 normal subjects above the age of 40 years were recruited . Age ,duration of disease and body mass index (BMI) was created. The mean levels of Smad1 and TRAIL- R2 in diabetics with sulfonylureas treatment , they were significantly higher compared to control subjects, while the means of SOD have low level in diabetic subjects , and the mean levels of TNF-R1 and IR were non-significant in diabetic with Sulfonylureas treatment compared to control subjects.

Keywords*: T2DM, TNF-R1, TRAIL- R2, Smad1, SOD , Sulfonylureas.*

Introduction

Diabetes mellitus (DM) was a metabolic disorder in which the body is unable to manufacture or use insulin adequately. Type 2 diabetes (T2DM) is a heterogeneous syndrome that was related to both defective secretion of insulin and insulin resistance(1). Poorly controlled levels of blood glucose usually lead to different complications in microvascular organs, such as nephropathy. (2) Diabetic nephropathy (DN) was a vascular disease risk factor and was widespread among T2DM patients (3). The request for more very reliable sensitive and specific biomarkers was needed for early expectation the progression and beginning of DN (4). Small mothers against decapentaplegic (Smad) signaling was Has been known as a main route of transforming Growth Factor- β (TGF- β) signaling in progressive fibrosis of renal (5). The Smad family of proteins was classified into three classes: Receptor-regulated Smads R-Smad (Smad1, Smad2, Smad3, Smad5, and Smad8), co-Smad (Smad4), and Inhibitor Smads I-Smad (Smad6 and Smad7).(6) Smad1was novel biomarkers in serum and urine for kidney injury associated with DN it can be used in the early diagnosis of DN or in the valuation of the treatment to prevent (7).Hyperglycaemia induces increase free radical formation .(8) A family of antioxidant enzymes were superoxide dismutase (SOD) that control reactive oxygen species (ROS) levels by catalyzing of the superoxide radical conversion to hydrogen peroxide and oxygen molecule (9) . Apoptosis was the natural mechanism of the cell for programmed cell death, it plays a vital role in both growth and homeostasis (10). Apoptosis can be induced by intrinsic pathways that are caused by cellular stress and DNA damage or by extrinsic signals that trigger death receptors on the cell surface. Such as TNF receptor 1 (TNFR-1) and ligand receptor 2 (TRAILR-2) linked to apoptosis-inducing TNF. (11)

Most patients with T2DM ultimately require pharmacologic glucose-lowering therapy, with a goal of decreasing long-term complications.(12)

Sulfonylureas have an excellent blood glucose reducing effect ,sulfonylureas was an effective drug for diabetes treatment and hyperglycemia (13).The current study was conducted to investigate the effect of Sulfonylureas as a monotherapy in diabetic patients on the release of soluble death receptor-activated apoptosis (TNFR/R1 and TRAIL/R2) , study the effect of Sulfonylureas drugs on activity of Smad1 in serum as a novel biomarker for early detection and evaluation of diabetic nephropathy severity in patients with T2DM (for the first time measured in blood serum) and Study the impact of Sulfonylureas on stress from oxidation by determination the activity of superoxide dismutase (SOD) . Thus to explore the effect of this treatment on the risk of developing diabetes complications related to kidney disease .

Materials and method

At the National Diabetes Centre / AL -Mustansiriya University thirty four (34) of T2DM patients and healthy subjects were chosen . The patients samples were divided to two groups. Group1 consists of 17 Sulfonylureas therapy taking patients and the group 2 was consists of 17 apparently healthy individual (control group). Patients with other diseases ,smoking patients and pregnant female patients were excluded from the medical assessment study. Patients that take other than metformin drugs or metformin plus insulin had also been excluded. he blood sample was split into two aliquots: the first aliquot blood was delivered to a tube comprising Ethylene Diamine Tetraactic Acid (EDTA), this blood was gently mixed and then used to measure HbA1c, while the second aliquot was delivered to the gel plain tubes to extract serum .Control and patients subjects were subjected to determination of the tests: serum insulin hormone was measured by.ELISA using kit (Sandwich) supplied by Cobas *(ROCHE),* the Homeostasis Model Assessment (HOMA) assessed insulin resistance (IR),SOD was determined in serum by (riboflavin/NBT method),TNF/R1 was measured using ELISA kit (Sandwich) supplied by Cusabio (USA), serum TRAIL/R2 was measured by using Sandwich ELISA kit provided by Cusabio (USA), serum Smad1 was measured by using ELISA kit (Sandwich) supplied by Mybiosource (CANADA) ,HbA1C % was measured using the Integrated Sciences(Australia) A1C test system, fasting plasma glucose concentration , serum urea and serum creatinine were estimated by colorimetric method by using a kit supplied by Bio Systems (SPAIN) .

Statistical Analysis

Statistics was performed with T-Independent test for difference between two groups ,the result were expressed as mean ± SD, different range test at $p \leq 0.05$ which was agreed as significant, where as highly significant when $p \leq 0.001$, and the range test at $p \geq 0.05$ was agreed as non- significant.

Results & Discussion

Demographic data for (17) patients with T2DM and (17) healthy subjects, the result in Table (1) showed that the Mean± SD value for study parameters, and Compare between different groups in current study.

Table (1): Distribution of mean± SD for all variables and parameters in all Studied Groups

Groups Parameter	G1=17 sample Diabetic with Sulfonylureas therapy	G1=17 sample Healthy control group	P-value
Age (years)	58.52±7.55	51.88±8.73	*0.032
Duration (years)	7.82±4.77	0.000	---
BMI (Kg/m2)	28.51±2.84	30.19±2.23	0.203
F.S.G.(mg/dl)	200.58±53.81	92.23±11.33	*0.000
HbA1C (%)	8.89±2.12	4.69±0.61	*0.000

Cont... **Table (1): Distribution of mean± SD for all variables and parameters in all Studied Groups**

Insulin (µU/mL)	12.49 ± 6.25	11.73 ± 4.27	0.240
IR	2.07 ±1.03	1.327±0.188	0.310
S. Urea (mg/dL)	29.54±6.84	23.7824±3.44	*0.045
Creatinine (mg/dL)	0.60±0.16	0.58±0.10	0.432
Smad 1 (ng/mL)	1.244±0.315	0.`144±0.026	*0.000
SOD (U/ mL)	109.68±13.65	151.91±11.64	*0.000
TNF-R1 (pg/mL)	0.563±0.205	0.555±0.220	0.392
TRAIL-R2 (pg/mL)	0.48±0.12	0.28±0.07	*0.00

*** Significant using ONEWAY-ANOVA at 0.05 level**

The results of **age** have showen that there were highly significant difference among the study groups , These results were due to the duration of the disease for the patient group. Note that all samples and healthy control were chosen above the age of 40 years old. **Body mass index BMI** showed no significant differences (P=**0.203**) between patients group, when they were compared to control group .

The statistical distribution of mean for glycaemic state (FPS, HbA1c , Insulin Level, and HOMA IR) were performed on all studied groups generally as shown in Table (1). *Results of fasting glucose in plasma* **(FPG)** expressed in mg/dl *revealed that there were high significant variations between patients group when compared to healthy control group(P<0.0001)* .The results of **glycated hemoglobin (HbA1c %)** levels showed higher significant increasing in patients group (*P<0.0001*), when compared to the control healthy subjects. In current study, a high level of HbA1c and FPG were found in patients group, which identifies to poorly controlled glycemia in these subjects.These result because of treating with Sulfonylureas he initial excellent response resulted in a loss of active antidiabetic response and induce βcell failure (secondary failure) (14) .The results of serum **insulin** levels (µU/mL) for patient groups showed that there were no significant differences between patients group when compared with control group. The same results have been observed in the insulin resistance levels in studied groups which indicated that there was no significant differences between patients group compared with control healthy subjects. The results of insulin and insulin resistance in our study were connected and intertwined .The results of current study were agreed with previous studies which explained that sulfonylurea drugs , showed some evidence to reduce insulin resistance in addition to its insulin secretory action. (15) Mean± SD for renal function tests (Urea, and Creatinine) were performed on all studied groups as shown in Table (1).The result of **urea** concentration (mg/dl) significant differences are observed in it is level of patient group compared to control healthy group (P=**0.045)** . All results are within normal values, Previous studies were showed that there was statistically significantly elevated levels of urea with increased levels of blood blood sugar and a strong connection between blood urea and blood sugar. [(16)]

Serum creatinine is a more accurate evaluation of renal function than urea, but urea has been increased in renal disease earlier. (17) The results of creatinine concentration (mg/dl) showed no significant differences are found in it is level of patients group compared to control healthy group.Serum urea and creatinine measures have been readily available tests that assist in

early diagnosis and avoidance of diabetic kidney disease and can restrict development to end-stage renal disease . (18) Smad1 were novel reliable biomarkers for each of the classical pathological finding in the beginning periods of DN and were imdicator of progressive glomulare filtration rate decline (19). The results of the Serum Smad1 concentration (ng/mL) ,which was measured for the first time in the blood serum, showed that there were highly significant differences between patients groups and healthy control subjects (*P<0.0001*). The mean± sd of Smad1 concentration in diabetic patients with sulfonylureas treatment was found to be significantly elevated compared with healthy subjects group, this results revealed that using of sulfonylureas as monotherapy in diabetic patients does not reduce the risk of high Smad1 concentration. The effect of sulfonylureas treatment was not recorded in previous studies , recent studies found The Smad1 is the primary ignaling molecule directly concerned in DN and other kidney disease induction and development of glomerulosclerosis (20) . The results of the serum total **SOD** levels (U/ mL) in T2DM patients and control groups showed that there were highly significant differences between studied groups (*P<0.0001*)**.**The mean± sd of **SOD** concentration in control group (**151.91±11.64**) was significantly increased compared with patients group (0.844 ± 0.285) . This finding was generally consistent with literature data and was a clear indicator of the presence of oxidative stress in people with diabetic . Pervious study showed that hyperglycaemia in diabetes stimulates different biochemical processes leading to an increase superoxide and hydroxyl radical formation, which may lead to reduced n SOD enzyme activity. (21), While some have not documented any changes(22) And in diabetic patients, few researchers found elevated serum SOD enzyme activity(23). Recently it was shown that death receptors can be established in a soluble form in the bloodstream (24). Levels of serum **(TNF-R1)** concentration (pg/mL) for studied groups showed that there were not significant differences between patients group when compared with control group , so the result of the current study showed that using of Sulfonylureas therapy may reduce **TNF-R1** concentration .This finding was consistent with literature data which know that the levels of serum TNF and its receptors were significantly decreased in diabetic patients with Sulfonylureas treatment (25). The results of **TRAIL-R2** concentration (pg/mL) showed elevated significant difference between patients groups (P<0.0001), when compared to control groups. This result correspond to study which showed that a high glucose level , characteristic of diabetes was increased serum TRAIL binding to TRAIL-R1 and TRAIL-R2 levels(26). Rresults of apoptosis factor (**TRAIL-R2) , and SOD** were generally consistent with literature data which showed that sulfonylureas treatment have been reported to accelerate cells apoptosis and stimulated production of reactive oxygen species (ROS) which could result in reduced activity of SOD enzyme. (27) These results may explain the high concentration of serum Smad1 in diabetic patients which indicates that patients with sulfonylureas treatment may exposure risk of developing diabetes complications related to kidney disease (Diabetic Nephropathy **) .**

Conclusion

Using of Sulfonylureas as a monotherapy in T2DM patients did not reduce the risk of developing diabetes complications related to kidney disease (Diabetic nephropathy**)**.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

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