Research Article

Influence of Sulfonylurea and Insulin on Immunological Profile of Type 2 diabetic Egyptian Patients

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Abstract

Objective: Type 2 diabetes mellitus (T2DM) is a chronic, inflammatory disease caused by long-term imbalance in immune system, metabolic syndrome, or nutrient excess associated with obesity .Sulfonylurea and exogenous insulin have been used in the treatment of T2DM, and they have hypoglycemic and anti-inflammatory effects .The aim of this study to demonstrate the effect of sulfonylurea and exogenous insulin on some immunological parameters in Egyptian patients with T2DM and determine whether diabetes or the type of treatment would influence the levels of these parameters.

Materials &Methods: This study was performed on 150 outpatients with type 2 diabetes matched with age and gender with 40 healthy subjects was selected from the outpatient's clinics of National Institute for Diabetes and Endocrinology. All studied patients and control were subjected to estimate Fasting blood glucose (FBG), Glycosylated heamoglobulin (HBA1c)**,** White blood cells (WBCs)**,** Interleukin-6(IL-6), Immunoglobulin G (IgG) and Immunoglobulin A (IgA).

Results: The levels of FBG, HBA1c, WBCs, IL-6, IgG and IgA showed highly significant increase in the diabetic patient groups compared to controls. ($P<0.001$).Treatment of T2DM patients with sulfonylurea and insulin caused highly significant decrease in the levels of FBG, HBA1c as compared to corresponding non -treated group ($P<0.001$). Also, the level of IL-6 revealed a highly significant decrease in insulin treated patients   as compared to corresponding non -treated group($p<0.001$).While, non-significant change ($p>0.05$) in the levels of WBCs, IgG and IgA was observed in treated patient groups with sulfonylureas and insulin as compared to corresponding non -treated group.

Conclusion: Both sulfonylurea and insulin are immune- safe therapeutic agent in T2DM at dose achieve good glycemic control.

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Introduction

Diabetes Mellitus (DM) is the five leading important diseases causing death globally and remains a major health problem in Africa [1]. Type 2 DM (T2DM) is the most common form of DM.T2DM is a chronic, inflammatory disease caused by long-term imbalance in immune system, metabolic syndrome, or nutrient excess associated with obesity [2]. Also, the complications of T2DM in the kidneys, arteries, and eyes are also manifested by inflammatory process [3]. Several studies have focused on the association between the immune system and T2DM [4, 5].Components of the immune system is deranged in T2DM [6]. The changes in immunology include altered in specific cytokines as IL-6, changed in numbers and activation states of various white blood cells populations, and immunoglobulins [7-9] .Thereby, optimal anti diabetic treatment needs beneficial effects that can help to prevent complications of diabetes, Moreover, achieving good glycemic control.

Most people who are newly diagnosed with type 2 diabetes are usually treated with a combination of diet, exercise, and an oral medication. Some oral medications (eg, metformin) improve the body's response to insulin. Other medications cause the body to produce more insulin. There are still a large number of people who are taking sulfonylureas (a class of drugs used in type 2 DM), either as a first-line DM treatment or in combination with another DM drugs. Glimepiride is a second-generation sulfonylurea that stimulates pancreatic β cells to release insulin; it may have potent anti-oxidative, anti-inflammatory and angiogenic properties [10].

Insulin therapy could be initiated in different conditions and should be considered in newly diagnosed T2DM when blood glucose is >300 mg/dL or HBA1C is >10% or if the patient has symptoms of hyperglycemia. Further, after metformin a double or triple therapy could be considered [11]. Beside many actions of insulin on the cell on the human metabolism level; it ameliorates inflammation through suppression of pro-inflammatory cytokines and immune mediators [**12**]. Although, numerous studies estimated the immunological profile in T2DM [5, 13, 14]; just a few studies have reported the immunological profile of T2DM patients in respect to type of treatment [15-17]. Therefore, the present study is undertaken to evaluate the impact of sulfonylureas and exogenous insulin on WBCs IL-6, IgG, and IgA in Egyptian patients with T2DM and determine whether diabetes or the type of treatment would influence the levels of these parameters.

Materials & Methods

Population

All subjects were collected from the outpatient clinic of National Institute of Diabetes and Endocrinology "NIDE", El -Kasr El-Einy Street, Cairo, Egypt and classified into 150 patients with T2DM and 40 healthy volunteers. Type 2- diabetes mellitus was diagnosed according to the American Diabetes Association Criteria. Blood samples were collected and questionnaire was performed. Approval had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes. An informed consent was obtained from all patients and healthy subjects.

*Inclusion and exclusion criteria included***:** 1) all patients were not taking any non-steroidal anti-inflammatory, aspirin and statin drugs, Angiotensin Converting Enzyme Inhibitor (ACEI) and anti-diabetic drug Thiazolidinedione (Glitazones). 2) They treated only with two injections of intermediate-acting insulin or sulfonylurea (glimepiride 2mg daily) for at least 1year. 3) None of them were complaining of any acute or chronic illness as confirmed by physical examination and laboratory testing results. The studied subjects were divided into 4 groups as following: group 1: Including 50 non- treated type 2- diabetes mellitus(recent diagnosed)(25 male, and 25 females, mean age50.1±7.5),group 2: Including 50 type 2 diabetic patients treated with sulfonylurea (glimepiride) (25 male, and 25 females, mean age52±7.1) , group 3: Including 50 type 2 diabetic patients treated with insulin (25 male, and 25 females, mean 51±8.1)and group 4: Including 40 healthy subjects (20 male, and 20 females, mean age 49±8.4)*.*

Clinical measurements

The following parameters were recorded for all patients: age, BMI, blood pressure. BMI was calculated as weight (kg) divided by height squared meter (Kg/m2) according to [18]. Blood pressure measured with a mercury sphygmomanometer. Hypertension was considered if (systolic/diastolic blood pressure (BP) ($\geq$140/$\geq$90 mmHg), or use antihypertensive agents.

Laboratory measurements

The levels of Fasting blood glucose (FBG), glycosylated haemoglobin (HBA1c) and White Blood Cells (WBCs) were assessed for all patients using classical methods. The levels of IL-6 concentration was measured using commercially available enzyme-linked immunosorbent assay (ELISA) Kit Immunodiagnostic (Orgenium, Inc. Tiilitie 3 FIN-07120 Vantaa, Finland), and immunoglobulins (IgG & IgA) were determined using Biotechnica3500 (BT 3500®) system by immune-turbid metric methods.

Statistical analysis

The obtained results were performed with Statistical Package for the Social Sciences (SPSS) software (version 11.5). Unpaired t-tests, and univariate (analysis of variance, ANOVA) were used to assess differences between groups. Pearson correlation coefficient (r) was used to measure the strength of the association between the variables .The results were expressed as means $\pm$standard deviation (SD). The difference at *p*- value less than 0.05 were considered statistically significant.

Results

The profile of characterization and clinical features of 150 patients and 40 control healthy individual were shown in Table 1.No significant differences were found between diabetic patient groups (group 1-3) and control (group 4) with respect to age and sex and (P> 0.05). While there was significant differences in Body Mass Index (BMI), systolic blood pressure (SBP) and, diastolic blood pressure (DBP) of diabetic patients as compared to control (P<0.05)**.**

The effect of administration of sulfonylureas and insulin on Fasting blood glucose (FBG), Glycosylated haemoglobin (HBA1c) was represented in Table 2. The level of FBG and HbA1c showed a highly significant increase in the diabetic patient groups (group 1-3) as compared to corresponding control group (group 4) (*p*<0.001). However, treatment of T2DM with sulfonylurea and insulin(group 2 and 3) showed a highly significant decrease in the level of FBG and HbA1c as compared to corresponding non-treated group (group 1) (*p*<0.001) (Table 2).

Also, in T2DM ,the level of White blood cells (WBCs), interleukin-6 (IL-6), immunoglobulin G (IgG) ,and immunoglobulin A (IgA) highly significant increase in the diabetic patients groups as compared to corresponding control group (*p*<0.001). While, after treatment of T2DM with sulfonylurea and insulin(group 2 and 3), the level of IL-6 showed highly significant decrease in insulin treated group (group 3) as compared to corresponding non-treated group (group 1) (*p*<0.001), and no significant difference observed in sulfonylurea treated group (group 2). Moreover, non-significant difference noticed in the levels of WBCs, IgG and IgA (*p*<0.05) in treated patient groups (group 2 and 3) as compared to corresponding non-treated group (group 1) (*p*>0.05) (Table 3).

Discussion

There is growing interest in the potential therapeutic benefits of anti-diabetic drugs available for the treatment of diabetes and therapeutic choices can be made on the basis of an expanding knowledge base of the

immunological safety of the available drugs. Our results revealed a higher significant increase in FBS and HbA1c levels in all patient groups (group 1-3) as compared to the control group (group 4), a high level of FBG and HBA1c may be attributed to abnormality in insulin producing cells (β cells of the Islets of Langerhans) and/or resistance of the system to insulin action[19]. However, treatment of T2DM with sulfonylurea or insulin caused a highly significant reduction in the level of FBG and HBA1c as compared to non- treated T2DM patients (group1) due to sulfonylurea and insulin achieved adequate glycemic control. This is in agreement with the results obtained by Eriksson *et al*. [20] and Inzucchi *et al*. [21].

White blood cells (WBCs) or leukocytes (a marker of inflammation) are cells of the immune system involved in defending the body against both infectious disease and foreign materials [22]. In the current study, WBCs were significantly increased in diabetic patient groups (group 1-3) as compared to control (group 4). This is consistent with other findings [23, 24] who suggested increased levels of WBCs in T2DM.Such results can be explained by hyperglycemia itself that has an impact on WBCs levels. Also, other result demonstrated that the high level of the WBCs is an indicator of worse disease state. The result showed an association between insulin resistance and WBCs [25]. A higher WBCs and insulin resistance reflected an underlying activation of the immune system. However, no significant alteration in WBCs was found neither in sulfonylurea treated patients (group 2) nor in insulin treated patients (group 3) as compared to non-treated patients (group 1) .These observations is contrast with other finding that demonstrated, some oral anti-diabetic drugs can cause leukopenia (the reduction in the number of white blood cells)[22]. Also, it was found that, WBC counts are lowered by treatment with rosiglitazone [26] which may be due to the lowering of glucose levels or an immunomodulatory effect of this drug. Similar decrease in WBCs has been observed with other types of anti-diabetic drugs including acarbose [27].

Production of pro-inflammatory cytokines is increased in patients with T2DM [28]. These include adipo cytokines such as IL-6 [30], which is a co-factor for immunoglobulin synthesis [29] and a common marker of inflammation [28]. Numerous studies have stated that several humoral markers of inflammation are elevated in people with T2DM. It was found that long-term activation of the innate immune system may be involved in the development of insulin resistance and T2DM [30]. This in accordance with our result in the study in which there is a highly significant increase in interleukin-6 (IL-6) in diabetic patients groups as compared to control. Similarly, many authors have reported higher levels of IL-6 are associated with the elevated risk of diabetes, supporting an association between chronic inflammation and development of diabetes in populations with different ethnical variations [32, 33]. However, these observations are contract with the findings of Al-Shukaili *et al*. who reported that the value of IL-6 was significantly decreased in T2DM as compared to control subjects [34].

Treatment of T2DM with sulfonylurea (group 2) showed no significant difference in the level of IL-6 in comparison to non -treated group (group 1). However, treatment of T2DM with insulin (group 3) caused a high significant reduction in the level of IL-6. These results are in accordance to work which suggested a decreased level of IL-6 in insulin treated patients than sulfonylureas treated patients [17]and disagreed with other previous findings [15,16]. This may be explained by the fact that insulin works as an anti-inflammatory agent directly through cellular effects, which might be by modulating glucose concentration. Also, it was found that insulin reduced mortality and prevented the occurrence of failure of multi-organ in critically ill subjects at a dose that kept blood glucose at normal level [35]. In an animal experiment, insulin had anti-inflammatory effects by reducing the levels of pro-inflammatory signal transcription factors and pro-inflammatory cytokines [36].

Serum immunoglobulin concentrations tend to increase with exposure to pathogens (antigens) [37].Moreover, immunoglobulin levels help in the diagnosis of some disorders, as liver diseases [38].The present study revealed that, the levels of immunoglobulinsG (IgG) was highly significantly increased in diabetic patient groups (group 1-3) as compared to corresponding control group (group 4). This may be explained by the fact that probable infection in the diabetic patients [5, 9]. These results are consistent with the work done in animal study that suggested increased IgG against specific bacterial antigens in diabetes [39].

On other hand, these results disagree with the results done by Guo *et al*. that demonstrated decreased level of IgG in T2DM and showed an inverse relationship with the prevalence of T2DM [9].

In our study, the levels of IgA highly significantly increased in all patient groups (group 1-3) in comparison to control (group 4). Several studies also showed that the higher concentration of IgA observed in patients with T2DM might

be due to the result of an immune response to advanced glycosylation end products, whose increase and accumulation are caused by persistent high blood glucose [9]. While treatment of T2DM with sulfonylureas or insulin, showed no significant alteration in the levels of IgG and IgA in sera of sulfonylurea and insulin (group 2-3) treated patients as compared to non-treated patients (group 1).

The ensemble of results discussed herein, could have considerable clinical consequences and contributes to prove that both sulfonylureas and insulin are "immune-safe "chemotherapeutic drug and hormonal-therapeutic agent in T2DM, respectively. Nevertheless, a lot remains to be clarified and prospectively assessed, but our data point to a crucial role of WBCs, IL6, IgG, and IgA as reliable predictive biomarkers that could be used to identify "Diabetic Candidates".

In conclusion, the level of immunological parameters (WBcs, IL-6, IgG and IgA) increased in T2DM.Treatment of T2DM patients with sulfonylurea or insulin remain effective way to lower blood glucose rapidly, although these forms of treatment haven't any effect on the levels of WBcs, IgG and IgA. The inflammatory level measured by IL-6 was improved by insulin therapy that was has anti-inflammatory property. Diabetes itself responsible for increased levels of these parameters, not type of treatment that used in this study.

Table 1 Profile of the investigated groups and their clinical features (M ±SD)

Parameters	group 1 (n=50)	group 2 (n=50)	group 3 (n=50)	group 4 (n=40)	p
Sex (M/F)	25/25	25/25	25/25	20/20	>0.05^{NS}
Age (years)	50.1 ±7.5	52 ±7.1	51 ±8.1	49 ±8.4	>0.05^{NS}
BMI (kg/m^2)	25.5 ±0.45	26.2 ±0.37	28.9 ±0.52	24 ±1.5	<0.05*
SBP (mmHg)	130 ±33.5	135 ±63.1	138 ±59.4	110 ±53.3	<0.05*
DBP (mmHg)	80 ±14	82 ±17	90 ±22	75 ±5.5	<0.05*
Duration of diabetes (years)	———	3.1 ±1.6	5.2 ±2.1	———	
Duration of treatment (years)	———	2.5 ±1.1	2.9 ±0.9	———	

Note: S.D.: standard deviation, **n:** Number of samples ***:** significant difference (**P**<0.05), **NS:** non-significant difference **P**> 0.05. Body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP). **P** :(group1-3 vs. group4)

Table 2 The level of Fasting blood glucose (FBG) and glycosylated hemoglobin (HBA1c) in diabetic patients groups with and without treatment with sulfonylurea and insulin (group1-3) and control group (group4) (M ±SD).

Parameters	group 1 (n=50)	group 2 (n=50)	group 3 (n=50)	group 4 (n=40)	P1	P2	P3
FBG (mg/dl)	291 ±70.7	151 ±12.5	156 ±9.2	91.7 ±8.5	<0.001**	<0.001**	<0.001**
HBA1c (%)	9.6 ±1.15	6.9 ±0.39	7.08 ±0.24	5.3 ±0.57	<0.001**	<0.001**	<0.001**

Note: S.D.: standard deviation, **n:** Number of samples. ** Highly significant (p<0.001).**P1** :(group1-3 vs. group4), **p2** :(group1 vs. group2), **p3:** (group1 vs. group3).

Table 3 the level of White blood cells (WBCs), interleukin-6 (IL-6), immunoglobulin G (IgG) and immunoglobulin A (IgA) in diabetic patients groups with and without treatment with sulfonylurea and insulin (group1-3) and control group (group4) (M ±SD).

Parameters	group 1 (n=50)	group 2 (n=50)	group 3 (n=50)	group 4 (n=40)	P1	P2	P3
WBCs (1000/cm³)	7.33±1.32	7.66±1.43	7.5±1.5	6.49±1.8	<0.05*	>0.05^{NS}	>0.05^{NS}
IL-6 (pg/ml)	26.8±13	25.9±8.2	8.16±2.2	2.36±0.7	<0.001**	>0.05^{NS}	<0.001**
IgG (mg/dl)	1909±494	1961±442	1891±477	926±147	<0.001**	>0.05^{NS}	>0.05^{NS}
IgA (mg/dl)	452±98	427±86	413±112	201±57	<0.001**	>0.05^{NS}	>0.05^{NS}

Note: S.D.: standard deviation, **n:** Number of samples. **NS:** non-significant difference ($P>0.05$), *****significant difference ($P<0.05$), ** highly significant ($p<0.001$). **P1** :(group1-3 vs. group4), **p2** :(group1 vs. group2), **p3:** (group1 vs. group3)
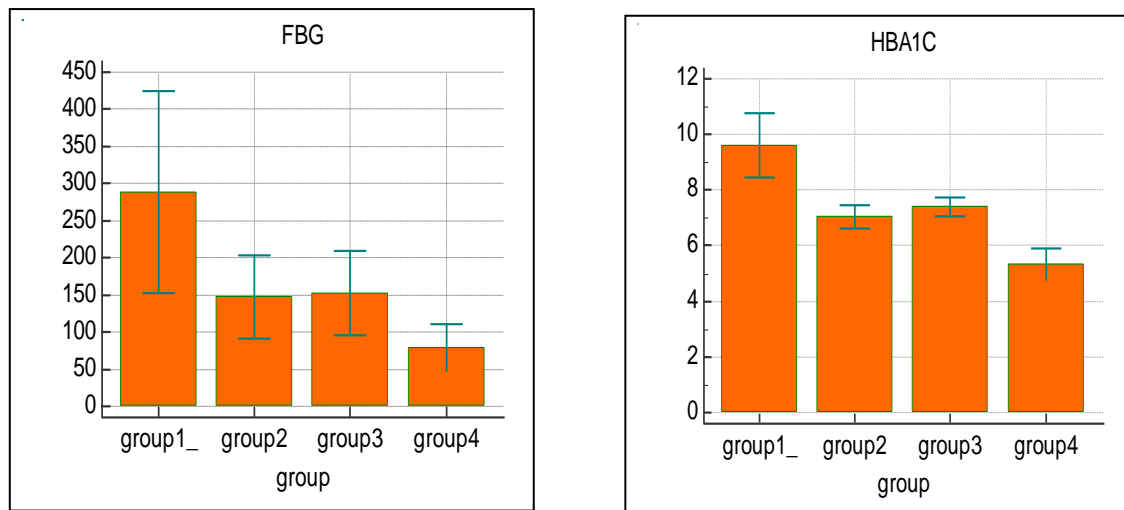

Figure 1 the level of Fasting blood glucose (FBG) and glycosylated hemoglobin (HBA1c) in diabetic patients groups with and without treatment with sulfonylurea and insulin (group 1-3) and control group (group 4)
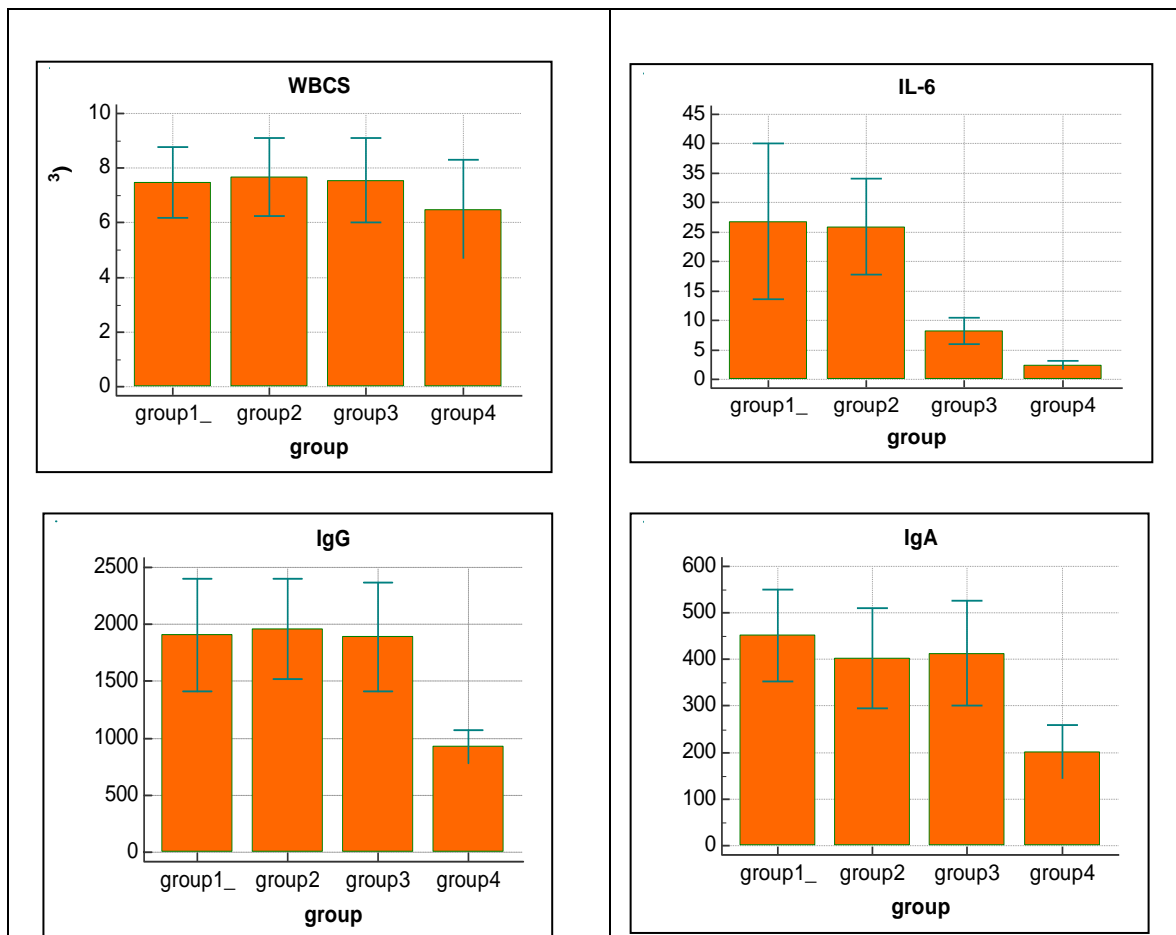
Figure 2 the level of White blood cells (WBCs), interleukin-6 (IL-6), immunoglobulin G (IgG) and immunoglobulin A (IgA) in diabetic patients groups with and without treatment with sulfonylurea and insulin (group1-3) and control group (group4)

Acknowledgement

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