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Effect of Pioglitazone on Glucose and Glycation Level in Type 2 Diabetic Patients

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ABSTRACT

To elucidate the effects of pioglitazone treatment on glucose and glycation level in patients with type 2 diabetes, a total of 8 patients were treated with pioglitazone (30 mg day^{-1}) for 12 weeks. After this period, there was a significant decrease in fasting plasma glucose and glycation level. The fasting plasma glucose was decreased from 195 to 159 mg per dL and glycation level decreased from 0.140 to 0.113 moles of glucose per moles of protein. These results suggest that pioglitazone decreases fasting plasma glucose in type 2 diabetic patients and acts as an inhibitor of glycation *in-vivo*. © 2010 Friends Science Publishers

Key Words: Pioglitazone; Glycation; Diabetic; Inhibitor

INTRODUCTION

Diabetes mellitus commonly referred to as "diabetes" is a group of metabolic diseases characterized by abnormalities at multiple organ sites. These defects include insulin secretion, insulin action or both (Barnett, 2007). There are different forms of diabetes mellitus but type 2 diabetes is the most common. It is also known as the non-insulin dependent diabetes mellitus (NIDDM) and occurs as a result of progressive insulin secretory defect on the background of insulin resistance (American Diabetes Association, 2009). About 90 to 95% of people suffering from this disease have type 2 diabetes (DeFronzo, 1997).

Without proper management many complications arise under hyperglycemic conditions. A high glucose concentration increases protein glycation, also known as non-enzymatic glycosylation in blood and various organs (Krishmanurti & Steffes, 2001). These non-enzymatic reactions between reducing sugars and free amino groups of proteins can alter the structure and function of proteins (Hartog *et al.,* 2007) and result in the formation of advanced glycation end products (Ulrich & Cerami, 2001).

Pioglitazone (±) 5-{[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl}-2, 4-thiazolidinedione is an oral anti-hyperglycemic agent, which is used in the treatment of type 2 diabetes (Gillies & Dunn, 2000). It belongs to a class "thiazolidinediones", represent a potentially important group of drugs with a mechanism of action differing from the existing therapies (Yki-Jarvinen, 2004). It acts primarily by decreasing insulin resistance in the treatment of type 2 diabetes (Radhakrishna *et al.,* 2002). It is a peroxisome proliferator activated receptor (PPARγ) agonist that increases transcription of insulin responsive genes and thus increases insulin sensitivity (Scholz *et al.,* 2001).

Most of the drugs metabolizing genes show different activities in different populations, which are often major determinants of variable drug exposure and response (Flockhart & Desta, 2009). Moreover individuals can differ greatly in their inherent capacity to absorb, distribute, excrete and metabolize drugs (Vesell, 1974). To accomplish the clinical benefits, safe and effective drug therapy is difficult due to large inter-patient variability in response to many drugs (Flockhart & Desta, 2009). The values of biochemical and physiological parameters present in the literature are not inline with the values calculated under our own environmental conditions. Therefore for the welfare of humanity it is very necessary to generate information in our population to develop our own therapeutic standards under local environment.

Pioglitazone has been studied rarely and its effect on glycation level has been reported only from few countries. Moreover glycation research remains an area of outstanding interest, fascination and innovation in chemistry, biology, medicine, food, nutrition and applied sciences. Therefore the present project was designed to widen our knowledge about pioglitazone (an anti-diabetic) in Pakistani patients under our own ecological conditions.

Study Design and Methods

Subjects: A total of eight type 2 diabetic patients, clinically diagnosed by physicians, were recruited from the outpatient medicine clinic of the General Hospital Faisalabad, Pakistan. They were recommended diet and exercise at initial stage to control the diabetes. Patients who had previously received insulin, metformin, thiazolidinedione or

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any other medicine related to diabetes were excluded. Entry criteria included age (35-55) years and weight (55-85 kg). Patients were in good general health without cardiac, hepatic, renal or other chronic diseases. The demographic and clinical data of the patients is presented in Tables I and II.

Study design: The study was conducted for 12 weeks by keeping in view the ethical principles laid down in the Declaration of Helsinki (World Medical Association, 2004). Initially subject's education was updated and their ability to comply with the protocol was checked. The subjects were instructed to take balanced diet during study period. All subjects gave signed, informed consent before participation in the study.

Plasma collection: Blood samples from each patient after every 15 days, up to 12 weeks were collected by using sterilize disposable syringes by venopuncture in pre-heparinised centrifuge tubes 10 mL Vacuette® Griener Bio-one, Austria. These samples were mixed gently and were then centrifuge at 4000 rpm and plasma was stored at –20°C.

Analysis techniques: In plasma samples amount of glucose was estimated by Kit method, protein concentration by Biuret method and glycation level by Thiobarbituric acid (TBA) method as described by Furth (1988), spectrophotometrically.

Statistical analysis: For computation and the graphics, the Microsoft Excel 7.0 was used. All data are reported as the mean±SE (Steel *et al.,* 1997).

RESULTS

The results illustrating the effect of pioglitazone on glucose concentration, proteins concentration and glycation level in diabetic patients are presented in the Figs. 1–5. In patients, there is regular decrease in the concentration of fasting plasma glucose (FPG). After 8th week, minimum concentration (159.0±3.30 mg dL^{-1}) was obtained in comparison to 195.5±8.65 mg per dL detected at the start of the study. This level slightly increased to 163.0±4.64 mg per dL after 10th week and then declined to 159.38±4.65 mg per dL (Fig. 1). There is not gradual increase or decrease in the concentration of plasma proteins. These values changed randomly and maximum concentration 67.96±1.77 mg per mL was achieved after 6th week and minimum value 65.83±1.93 mg per mL after 10th week (Fig. 2).

In patients, long term therapy with pioglitazone significantly decreased the glycation level. This level reduced gradually up to 12th week, but in last weeks value of glycation level become consistent. Maximum glycation level (0.144±0.014 mole $mole^{-1}$) was observed after 2nd week and minimum value 0.113±0.010 mole per mole was obtained after 12th week (Fig. 3). The trends between the change in fasting plasma glucose and glycation level and the change in concentration of proteins and glycation level are shown in Figs. 4 and 5, respectively.

Table I: Demographic data of diabetic patients

Subject		Age	Weight	Height	Blood Pressure mmHg		Body Temp.	BMI*
No.	ID	Years	Kg	Inches	Systolic	Diastolic	°F	Kg/m^2
1	A_P	42	74	66	134	82	98.5	25.62
2	B_P	45	68	58	130	72	97.8	31.33
3	C_P	49	82	66	140	85	99.2	29.18
4	D_P	38	71	59	124	78	98.2	31.61
5	E_P	53	80	67	129	84	98.4	26.24
6	F_P	50	81	60	136	90	98.5	34.87
7	G_P	43	76	62	126	86	98.7	30.65
8	H_P	36	70	63	122	84	98	27.34
Mean		44.5	75.25	62.63	130.125	82.625	98.4	29.61
± SE		2.24	2.02	1.31	2.34	2.07	0.16	1.18

Subscript 'P' stands for patient, *BMI is Body Mass Index

Fig. 1: Change in concentration of glucose in diabetic patients

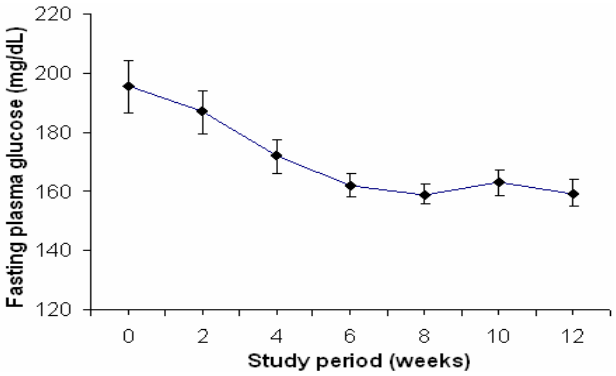
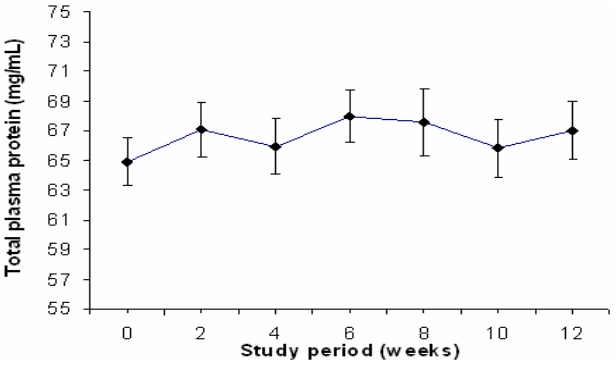


Fig. 2: Change in concentration of plasma proteins in diabetic patients



DISCUSSION

Results indicate that long term therapy with pioglitazone have significant effect on FPG level in type 2 diabetic patients. This effect is more pronounced up to 8th week then concentration become almost constant. These results are similar to the results reported in the literature. Pioglitazone decrease blood glucose in diabetic animal models (Saltiel & Olefsky, 1996) and in patients with type 2 diabetes (Suter *et al.,* 1992). In general decrease in plasma glucose or improvement of insulin resistance by pioglitazone requires several days in diabetic animals

Table II: Clinical data of diabetic patients in the fasting state

Subject		Glucose	Serum creatinine	Triglycerides	Hb	Cholestrol	SGPT	Total protein	TLC	Neutrophils	Lymphocytes
No.	ID	mg dL^{-1}	mg dL^{-1}	mg dL^{-1}	g dL^{-1}	mg dL^{-1}	U L^{-1}	g dL^{-1}	/cmm	%	%
1	A_P	192	0.7	125	13.5	174	30	7.2	7900	59	32
2	B_P	202	0.8	118	12.9	230	26	6.2	6500	57	36
3	C_P	224	0.5	235	13	192	35	6.5	8200	65	30
4	D_P	168	0.9	128	12.4	234	18	6.1	10100	58	35
5	E_P	182	1.1	171	11.4	160	41	6.2	7600	62	34
6	F_P	229	0.6	237	14.2	184	29	6.3	9200	57	32
7	G_P	157	1.0	323	15.1	142	21	6.6	9800	64	28
8	H_P	199	0.8	128	13.4	265	26	7.3	8700	60	34
Mean		194.1	0.8	183.1	13.2	197.6	28.3	6.6	8500	60.3	32.6
± SE		9.5	0.1	28.2	0.4	15.8	2.8	0.2	451.9	1.2	1.0

Subscript 'P' stands for patient, Hb**:** hemoglobin, SGPT: Serum Glutamic-Pyruvic Transaminase, TLC: Total leucocyte count

(Saltiel & Olefsky, 1996) or several weeks in patients with type 2 diabetes (Iwamoto *et al.,* 1991; Suter *et al.,* 1992). Aronoff *et al.* (2000) mentioned that the blood glucose lowering effect of pioglitazone developed gradually over weeks and maximum decrease was observed after 10-14 weeks. Pioglitazone have the ability to reduce both fasting and postprandial glucose levels (Suter *et al.,* 1992; Ravikumar *et al.,* 2008). After pioglitazone treatment, fasting and postprandial endogenous glucose production (EGP) decreased (16.6±1.0 vs. 12.2±0.7 µmol kg^{-1} min^{-1}) and (2.58±0.25 vs. 1.26±0.30 µmol/Kg/min), respectively (Ravikumar *et al.,* 2008). Rubin *et al.* (1999) also observed a decrease in FBG levels of up to 60 mg/dL in a combination therapy. The results about the analysis of plasma proteins illustrate that there is not regular increase or decrease in the concentration of proteins. These values remain within the normal limits. This indicate that long term treatment with pioglitazone have no effect on plasma proteins in type 2 diabetic patients.

The decrease in the glycation level after 12 weeks treatment with pioglitazone is not surprising, because similar results are available in previous literature. As the pioglitazone significantly improves the glycemic control (Pavo *et al.,* 2003; Umpierrez *et al.,* 2006), therefore its inhibitory effect on glycation process can be explained in this scenario. As monotherapy, it improves the fasting blood glucose and glycosylated hemoglobin (Stratton *et al.,* 2000; Pavo *et al*., 2003). On average, fasting blood glucose and glycosylated hemoglobin can be improved by approximately 40 mg per dL and almost 1%, respectively (Stumvoll & Haring, 2002). It was effective in reducing plasma glucose from the baseline levels from week 4 and this decrease continued up to the last measurement at week 12 (Abe *et al.,* 2007). In Wistar fatty rats, GHb levels declined gradually over 5 weeks administration of pioglitazone (Nagisa e*t al.,* 2003). It also acts as an important inhibitor of glycation and potent antioxidant (Rahbar *et al*., 2000; Gumieniczek, 2005). Moreover the pattern between the change in FPG and glycation level is quite similar, they show almost similar trends during the study. The pattern between change in concentration of proteins and glycation level in patients demonstrate that trend in the concentration change of plasma proteins is not similar to change in glycation level.

Fig. 3: Change in Glycation level in diabetic patients

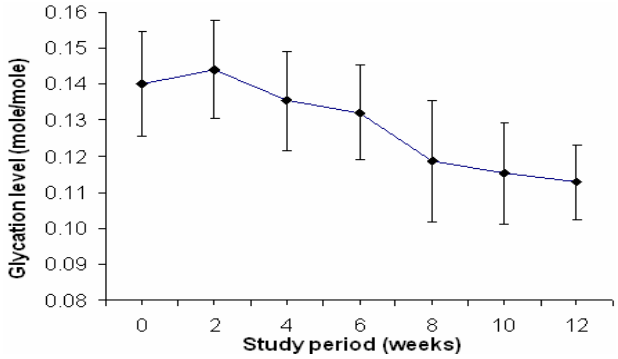


Fig 4: Pattern between glycation level and concentration of glucose in diabetic Patients

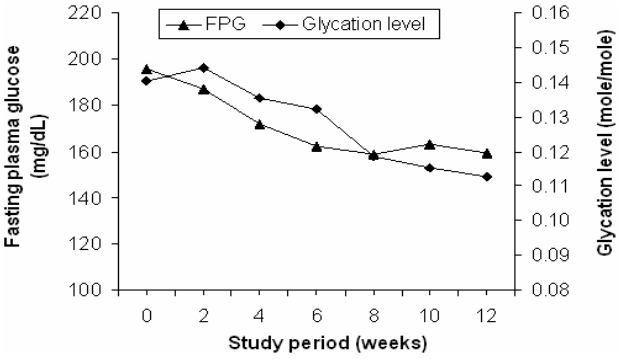
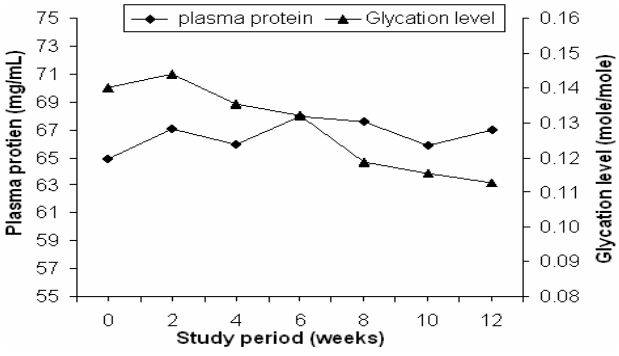


Fig. 5: Pattern between glycation level and concentration of proteins in diabetic patients



CONCLUSION

Pioglitazone decreases the fasting plasma glucose level and acts as an inhibitor of glycation in type 2 diabetes patients.

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