British Journal of Pharmaceutical Research
11(3): 1-9, 2016, Article no.BJPR.25480
ISSN: 2231-2919, NLM ID: 101631759

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Primary Unresponsiveness to Pioglitazone is not Related to PPAR-ϒ and Its Co-activator Gene Exon SNP Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Author PM managed the literature searches, collected all patient information and carried out experiments. Author PP collected patient information, compiled and analyzed the data. Author SL did statistical analyses of the study. Author MKM designed and guided the genotyping. Author MM coordinated and guided all the work. Author SKM designed the study, carried out clinical work and wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/25480
Editor(s):
(1) Abu Syed Md. Anisuzzaman, Winship Cancer Institute, Emory University, Atlanta, USA.
Reviewers:
(1) Rajendra Nath, King George's Medical University, Lucknow, India.
(2) Mustapha Diaf, Djillali Liabes University Sidi-bel-Abbes, Algeria.
Complete Peer review History: http://sciencedomain.org/review-history/14097

Short Research Article

Received 4th March 2016
Accepted 28th March 2016
Published 9th April 2016

ABSTRACT

The objective of this prospective observational study was to assess inter-individual variations in lipid and glycemic response to pioglitazone (30 mg OD) and their predictors in newly diagnosed T2DM patients. Out of 104 patients recruited, 88 completed 12 weeks follow up and were included in the

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final analysis. The patient characterstics studied were: BMI, W: H ratio, HbA1c, fasting and post meal glucose, lipid profile, HOMA-R, HOMA- beta and high resolution melting curve analysis (HRM) of PCR amplified DNA fragments flanking SNP markers of PPAR- γ and its co-activator gene. The mean decrease in HbA1c observed was 2.61% with wide inter-individual variation (coefficient of variance 66.44%, 95.75%, and 75.22% respectively for FPG, 2 hours PPG and HbA1c). Positive association between decrease in glycemic parameters and baseline HOMA- β, FPG, PPG HbA1c1 was observed. This finding suggest better glycemic response could be expected in those with higher beta cell function and severe hyperglycemia. Though lipid parameters improved significantly, they did not show any association with baseline characteristics studied as well as change in glycemic parameters. Eighteen (20.45%) patients were primary non-responder for glycemic, lipid and weight changes. This unresponsive could not be predicted on the basis of clinical and genetic parameters studied.

Keywords: Pioglitazone 1; Type 2 diabetes mellitus 2; glycemic response 3; PPAR-γ and is co-activator gene 4; Exon SNP markers 5; primary un-responsiveness 6.

ABBREVIATIONS

OD : Once a day
TZD : Thiazolidinedione
T2DM : Type2 diabetes mellitus
FPG : Fasting plasma glucose
PPG : Postprandial plasma glucose
HbA1c : Glycated hemoglobin
HOMA-R : Homeostatic model assessment of insulin resistance
HOMA-β : Homeostatic model assessment of β cell function
HRM : High resolution melting curve analysis
PCR : Polymerase chain reaction
DNA : Deoxyribose nucleic acid
ADA : American Diabetes Association
SNP : Single nucleotide polymorphism

1. INTRODUCTION

Pioglitazone is a thiazolidinedione (TZD) and has been widely used for the treatment of type 2 diabetes mellitus. It improves glycaemic control and appears to have some beneficial effects on lipid metabolism at the cost of weight gain. The TZDs improve insulin sensitivity by activation of peroxisome proliferator-activated receptor-gamma (PPAR- γ), primarily in adipose tissue [1]. The PPAR-γ has been the focus of intense research during the past decade because it is essential for adipogenesis and metabolic homeostasis. Besides its critical role in metabolic homeostasis, PPAR-γ modulates several cellular responses involved in atherothrombosis [2]. Ligand binding of pioglitazone to PPAR- γ causes adipocyte differentiation. Preclinical gain-of-function models show increased numbers of adipocytes and expansion of fat mass; loss-of-function models demonstrate lipodystrophy [3].

Through the mechansism of adipocyte differentiation, PPAR-γ activation promotes uptake of circulating fatty acids into fat cells. The consequent shifting of ectopic lipid stores from extra-adipose sites to adipose tissue promotes insulin sensitivity in target organs and contribute to glycemic and lipid effects of glitazones. The increase in fat mass, in conjunction with fluid retention leads to weight gain and it has been observed in many clinical trials [4]. The effect of thiazolidinedione on lipid profile is mixed and differs for individual compounds. Pioglitazone has shown to reduce triglycerides and raise HDL, whereas the effect on LDL remains inconclusive [5]. Growing evidence supports an array of additional effects of thiazolidinedione therapy, both immunomodulatory and anti-inflammatory, which may attenuate atherogenesis in type 2 DM [6].

Besides issues of weight gain, fluid retention and peripheral edema, primary unresponsiveness and inter-individual variations in response are major limitation of this drug. It is also not certain that what patient characteristics could predict better glycaemic response and primary

unresponsiveness to pioglitazone. In a previous study we find that primary unresponsiveness to pioglitazone is not related to common Pro12Ala polymorph of PPAR- γ gene [7]. Could it be related to some other sequence polymorphy of PPAR- γ gene, however remains unknown. Therefore the objectives of present study were to study (1) glycemic response to pioglitazone, (2) inter-individual variations in this responses, and (3) relationship between patient's clinical, biochemical characteristics and PPAR- γ gene sequence polymorphs and primary unresponsiveness to this drug.

2. MATERIALS AND METHODS

2.1 Subjects and Protocol

The present study was a prospective observational study conducted on newly diagnosed Type-2 DM patients reporting at a tertiary care hospital in India. Treatment naïve type 2 DM patients, who were prescribed pioglitazone, were enrolled in this study. The Institutional Ethics Committee approved the study protocol and written informed consent was obtained from all the subjects who participated in this study.

From June 2008 to Dec 2010, one hundred and four treatment naïve Type 2 DM patients diagnosed as per American Diabetes Association criteria (ADA, 2006) [8], with fasting plasma glucose > 130 mg/dl, and HbA1c > 7% despite adequate trial of medical nutritional therapy, were included in the study. The other inclusion criteria were BMI < 30 and no contraindication to pioglitazone. All patients were prescribed pioglitazone 30 mg per oral once daily. All the subjects were prescribed standard diet, physical activity and behavior therapy. They were counseled to strictly adhere to the prescribed diet and physical activity advice.

2.2 Clinical and Biochemical Evaluation

Detail patient characteristics were recorded on the first visit which included clinical history, physical and biochemical parameters and all patients were followed up for at least 12 weeks to assess the response to pioglitazone monotherapy. All the parameters were reassessed after 12 weeks and included for the final analysis. Subjects not responding to the monotherapy after 12 weeks of the treatment were switched to the other drugs as a rescue measure.

The study parameters were: BMI, waist circumference, W: H ratio, HbA1c, fasting and 2 hour post meal glucose, lipid profile, HOMA-R, HOMA- beta. Blood samples were collected after an overnight fast of at least 14 hrs. Blood was allowed to clot and the separated serum was used to measure the following biochemical parameters: FPG, PPG, lipid profile (phospholipids, triglycerides, total cholesterol, LDL, HDL, and VLDL), SGOT, SGPT and serum creatinine. These biochemical parameters were measured on Kopran AU/400 fully automated analyzer. Fasting serum insulin was estimated by chemiluminescence immunoassay using Immulite 2000 machine. HbA1c was measured by turbidimetry method.

Some parameters were estimated from initial general characteristics (1) Body mass index (BMI), (2) Waist: Hip ratio, (3) HOMA-R, (4) HOMA- B. The ratio of waist and hip circumference was used as an index of central obesity.

Insulin resistance was measured with Homeostasis model using the formula [9].

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

Glucose in mass units mg/dL

Beta cell function was measured with Homeostasis model using the formula [9].

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin}}{\text{Glucose} - 63}\%$$

Glucose in mass units mg/dL

2.3 Definitions of Glycemic Responders

Patients were separated into two groups: Responders and Non-Responders in terms of glycaemic response to pioglitazone. With respect to the definition of glycaemic responders, subjects with more than 10% decrease in their baseline HbA1c levels or achieving glycemic targets (ADA recommended target of glycemic control measures HbA1c < 7%) after 12 weeks of pioglitazone treatment, were labelled as responders.

2.4 Genotyping for PPAR- Gene and Its Co-activator SNP Polymorphism

Blood samples were collected in an EDTA tube and were stored at -80℃. Genomic DNA was

isolated from the blood samples using QIAamp kits using manufacturers protocol. The quality & suitability of extracted DNA was ascertained by determining the absorbance ratio 260/280 (nearest to 1.8) using NanoDrop spectrophotometer. The DNA segments containing SNP's of PPAR-γ (ENSG00000132170) gene region and its co-activator (ENSG00000112584) were amplified with following 10 primer sets (Table 1). For amplification of the specified region of PPAR γ gene, the reaction volume of 50 µl was prepared using 1X Taq buffer, 3 mM of $MgCl_2$, 200 µM of dNTPs, 5 pmol of each forward and reverse primer, 1 U of Taq polymerase enzyme (stratagene) and 50-100 ng of isolated genomic DNA. PCR was carried out on a PCR machine (M J Research Inc. PTC 200 Peltier thermal cycler). These amplified copies of DNA were then subjected to High Resolution Melting Curve Analysis (HRM), to detect mutations in the gene sequences of PPAR-γ gene. High Resolution Melting curve analysis was performed on The LightCycler 480 Real-Time PCR System of Rosche Applied Sciences. Following to PCR, the amplified region (amplicon) of PPAR γ gene was further processed for HRM analysis. Where the samples were heated to high temperatures (50℃ to 95℃), to denature the DNA (separating the two strands of DNA), and generating a melting curve. These melting curves were analysed to view, compare and detect the different genetic sequences.

2.5 Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 10.0.

3. RESULTS

Out of 104 patients initially recruited, 88 patients completed at least 12 weeks of follow up. Majority of the subjects were in fourth and fifth decade of life. Their mean age was 47.97 years with SD of 9.29 years. Male to female ration was 68:20.

3.1 Baseline General Characteristics

All the patients were non obese (BMI<30), with mean BMI (body mass index) at baseline 24.01±2.68 kg/cm^2. The mean waist circumference and W: H ratios at baseline were 89.662±8.36 cms and 0.96±0.09 respectively. The baseline HOMA- β and HOMA- R were respectively 14.94±12.18 and 2.82±1.93. The other baseline glycemic and lipid parameters are shown in Table 2.

3.2 Response to Pioglitazone Therapy

Table 2 shows the comparison of general characteristics and biochemical parameters at baseline and after the treatment. A significant change in the glycemic and lipid parameters was observed after the 12 weeks of treatment with pioglitazone. Mean reduction in FPG, PPG and HbA1c were 82.59 mg/dl, 125.45 mg/dl, 2.61% respectively. ADA's glucose control targets (FPG <130 mg/dl and HbA1c < 7 %) were achieved in 60(68.18%) and 38(43.18%) patients respectively. A mean reduction in total lipids (46.42 mg/dl) triglyceride (23.20 mg/dl), total cholesterol (14.98 mg/dl), LDL (14.76 mg/dl), VLDL (2.17 mg/dl) levels were observed. Increase in HDL (3.17 mg/dl) levels, weight (2.26 kg) and BMI (0.87 kg/m^2) was observed. Two patients (2.27%) out of 88 developed generalized edema. None of the participants developed congestive heart failure or reported any side effects other then weight gain and edema. There was wide inter-individual variation in glycemic response (coefficient of variance was 66.44%, 95.75%, and 75.22% respectively for FPG, PPG and HbA1c). Eighteen patients were primary non-responders.

3.3 Determinants of Glycemic Response to Pioglitazone

Multiple regression analysis of reduction in HbAc and various study parameters before starting treatment are depicted in the supplementary Table 1. Reduction in HbA1c showed statistically significantly positive correlation with HOMA-β (p 0.0484), HbA1c (p 0.0001) and FPG (p 0.0072) at baseline. There was a weak (borderline significance level) inverse correlation between the reductions in HbA1c and Waist to hip ratio (p 0.054) and HOMA-R (p 0.057) at baseline.

3.4 Determinants of Lipid Response to Pioglitazone

The relationship between reduction in lipid parameters and various study parameters before starting treatment is depicted in the supplementary Table 2. Except weak correlation between change in total cholesterol and W: H ratio (r 0.2447, p <0.05), there was no

association between baseline parameters and lipid changes. The supplementary Table 3 shows relationship between changes in glycemic parameters and change in lipid parameters.

Table 1. List of primer sets (forward and reverse primer)

S.N	Code (F)	Forward primer	Code (R)	Reverse primer
1	ENSGF1	CGCAAGGAACTCTGAAAGTG	ENSGR1	TTTCTCTTCTGATCGGTGGT
2	ENSGF2	GTTTCAGGATGATTCTTGTCTC	ENSGR2	GATTCATGCAGATCAAGATCAC
3	ENSGF3	CAATAAGCCTCATGAAGAGCCT	ENSGR3	CTTTATCTCCACAGACACGAC
4	ENSGF4	AAATGACAGACCTCAGACAG	ENSGR4	TGTCTCCGTCTTCTTGATCAC
5	ENSGF5	ACACGATTTGCTCTAAAGAC	ENSGR5	CTACCTCATAATCTGCTTCC
6	ENSGF6	GAATCCAGGCGAGAAGTTCC	ENSGR6	AACTTCTTGCCTGGGTTCAG
7	ENSGF7	TTTAGTCAACAGCGCATGTG	ENSGR7	GCATGAAATCACTCGTCTTCC
8	ENSGF8	GCTCACCAAATTCCACAACC	ENSGR8	CGTCTGTTCTCCTTCATGCA
9	ENSGF9	GCAGGAAGCAGACAGTATGAG	ENSGR9	TGACTAGTACCTTCTCCACTG
10	ENSGF10	CTTGCATGAAGAAGGAACGA	ENSGR10	ACAGAATCAGGCACAAGCTC

Table 2. Response to pioglitazone after the treatment in all the patients

	Baseline values (Mean±SD)	After treatment (Mean±SD)	t calculated	p value
BMI (kg/m^2)	24.01±2.68	24.88±2.91	5.661	< 0.001 †††
FPG (mg/dl)	212.84±55.91	130.25±40.92	12.027	< 0.001 †††
PPG (mg/dl)	323.36±91.37	197.91±85.29	10.329	< 0.001 †††
HbA1c (%)	10.26±1.82	7.66±1.64	11.033	< 0.001 †††
Total Lipids (mg/dl)	658.14±169.76	611.72±144.84	3.174	0.002077††
Phospholipids (mg/dl)	211.71±40.81	199.35±38.07	2.885	0.004933††
Triglycerides (mg/dl)	180.11±114.55	156.91±91.04	2.716	0.007962††
Total Cholesterol (mg/dl)	197.48±42.03	182.50±39.92	3.299	0.001405††
HDL (mg/dl)	44.81±7.07	47.99±7.86	3.275	0.001516††
LDL (mg/dl)	116.39±35.91	101.63±32.96	3.628	0.00048†††
VLDL (mg/dl)	36.41±23.09	34.24±31.45	0.6979	0.487119
Triglycerides/HDL	4.26±3.36	3.41±2.18	3.805	0.000263†††

$^{\dagger}p < 0.05$ significant, $^{\dagger\dagger}p < 0.01$ very significant, $^{\dagger\dagger\dagger}p < 0.001$ extreme significant

Table 3. Mean values of glycemic and lipid parameters before and after the treatment among responders (n=70) and non-responders (n=18)

Parameter	Responder / Non-responder	Baseline Mean ± SD	After treatment Mean ± SD	p value
HbA1c (%)	Responder	10.53±1.83	7.21±1.30	0.0001 †††
	Non-Responder	9.22±1.39	9.38±1.73	0.4709*
FPG Mg/dl)	Responder	219.93±54.54	119.87±30.89	0.0001 †††
	Non-Responder	185.28±53.99	170.61±50.30	0.0934*
P.P. Plasma Glucose	Responder	330.50±90.18	177.84±71.92	0.0001 †††
(mg/dl)	Non-Responder	295.61±93.23	275.94±90.15	0.2529*
Weight (kg)	Responder	65.34±9.59	68.24±9.45	0.0001†††
	Non-Responder	65.44±11.96	65.22±11.95	0.7659*
BMI (kg/m2)	Responder	23.81±2.41	24.91±2.67	0.0001†††
	Non-Responder	24.79±3.54	24.75±3.78	0.8605*
Total Lipids (mg/dl)	Responder	655.25±181.71	600.04±134.48	0.0021††
	Non-Responder	669.36±115.32	657.14±176.67	0.6140*
Triglycerides (mg/dl)	Responder	175.45±117.65	145.44±73.54	0.0044††
	Non-Responder	198.21±102.63	201.53±133.47	0.7765*
Total Cholesterol	Responder	199.24±44.20	179.93±40.68	0.0001††
(mg/dl)	Non-Responder	190.63±32.38	192.48±36.15	0.8794*
HDL (mg/dl)	Responder	44.67±7.04	47.64±8.19	0.0089††
Mean ± SD	Non-Responder	45.37±7.35	49.33±6.47	0.0715*
LDL (mg/dl)	Responder	118.38±37.29	101.30±34.64	0.0003††
	Non-Responder	108.65±29.62	102.91±26.19	0.5582*

**p > 0.05 not significant; $^{\dagger}p < 0.05$ Significant, $^{\dagger\dagger}p < 0.01$ Very significant, $^{\dagger\dagger\dagger}p < 0.001$ Extreme significant. All values expressed as Mean ± SD*

Except a weak positive relation between decrease in HbA1c and total cholesterol (r -0.2493, p <0.05), no association between changes in lipid and glycemic parameters were observed.

3.5 Responders vs. Non-responders (Clinical & Biochemical Parameters)

The patients were sub-divided as responders (n=70) and non-responders (n=18) and analyzed further. As shown in Table 3, among the responders there was not only a significant reduction in glycemic parameters (FPG, PPG, HbA1c, p <0.0001 for all) but there was significant decrease in total lipids (p 0.0021), phospholipids, triglycerides (p 0.0044), total (p <0.001) and LDL cholesterol (p <0.003). Responders also show significant increase in BMI (p <0.0001) and HDL cholesterol (p <0.0089). Therefore the primary unresponsiveness to pioglitazone is not only restricted to its glycemic response but also to its weight and lipid effects.

On comparison of general characteristics, lipid and glycemic parameters between responders and non responders, it was revealed that though non responders had marginally higher HbA1c (p 0.0055), FPG (p 0.0181) and HOMA- β (p 0.0274) most of other parameters were comparable in both the groups (Table 4). Though declining beta cell function is known to be associated with unresponsiveness to most of the oral anti-diabetic drugs But in the present study primary non-responders had higher HOMA-β and comparable HOMA-R, hence neither poor beta cell function nor insulin resistance could explain primary pioglitazone failure. Therefore primary unresponsiveness to pioglitazone could not be predicted on the basis of these parameters.

3.6 Responders vs. Non-responders (Genotyping for PPAR- gene and its Co-activator SNP Polymorphism)

On HRM analysis of DNA fragments flanking 10 SNPs spanning PPAR γ gene and its co-activating we find the exon 1 flanking SNPs was highly polymorphic among responders but not among non responders. But there was overlapping of melting temperature of responders and non-responders. Therefore it cannot be predicted that primary pioglitazone failure can be because of polymorphism of these SNP and flanking region.

Table 4. Comparison of patient characteristics among responders (> 10% decrease in HbA1c) and non-responders (<10% decrease in HbA1c)

	Responders (More than 10% decrease in HbA1c) n=70	Non-Responders (Less than 10% decrease in HbA1c) n=18	p value
Age	48.24±9.12	46.89±10.09	0.5841
Height	165.48±9.37	162.27±10.98	0.2150
Weight	65.34±9.59	65.44±11.96	0.9697
BMI	23.81±2.41	24.79±3.54	0.1661
Waist Circumference	35.37±3.12	35.19±3.99	0.8427
W: H	0.95±0.07	0.98±0.15	0.2544
Subscapular	29.29±7.46	28.44±10.44	0.6967
Fasting Insulin	5.12±3.39	6.53±4.50	0.1437
FPG at baseline	219.93±54.54	185.28±53.99	0.0181†
PPG at baseline	330.5±90.18	295.61±93.23	0.1496
HbA1c at baseline	10.53±1.83	9.22±1.39	0.0055††
HOMA-β	13.50±11.32	20.56±14.03	0.0274†
HOMA-R	2.73±1.76	3.20±2.52	0.3634
Total Lipids	655.25±181.71	669.36±115.32	0.7551
Phospholipids	213.2±42.80	205.93±32.29	0.5035
Triglycerides	175.45±117.65	198.21±102.63	0.4553
Total Cholesterol	199.24±44.20	190.63±32.38	0.4415
HDL	44.67±7.04	45.37±7.35	0.7126
LDL	118.38±37.29	108.65±29.62	0.3082
VLDL	35.58±23.75	39.61±20.59	0.5121
Triglycerides/HDL ratio	4.17±3.50	4.59±2.81	0.6391

†p < 0.05 Significant, ††p < 0.01 Very significant, †††p < 0.001 Extreme significant

4. DISCUSSION

The present study was undertaken to assess (1) inter-Individual variations in glycemic response to pioglitazone (2) its relation with baseline clinical characteristics of the patients and (3) predictors of primary unresponsiveness to this drug. A significant change in the glycemic and lipid parameters was observed after the 12 weeks of treatment with pioglitazone in the majority of subjects. Significant increase in BMI and HDL cholesterol was observed. All other lipid and glycemic parameters showed significant decrease. There was wide inter-individual variation in glycemic response. Eighteen patients were primary non-responders for glycemic, lipid and weigh changes and this un-responsiveness could not be predicted on the basis of studied patient characteristics.

In the analysis of all subjects, the average decrease in HbA1c levels observed in this study was 2.61 percent point. This decrease in HbA1c is much more than what has been reported for Caucasians. With the same dose of pioglitazone Aronoff et al. [10] and Herz et al. [11] reported respectively 1.3 and 0.8 percentage point decrease in HbA1c. However, in another study from south India, using the same daily dose of pioglitazone Ramchandran et al. [12] observed average HbA1c reduction of 2.6%, a finding similar to the result of present study on Indian population. One explanation of the higher glycemic response among Asian Indians is their lean high insulin resistance phenotype [13-15]. Poor calorie storage capacity (i.e. adipogenesis) in subcutaneous adipose tissue is one of proposed mechanism of Asian Indian lean high insulin resistant phenotype [16]. Findings of our ongoing study on transcriptomics of adipose tissue where adipogenesis was found to be down regulated in subcutaneous adipose tissue of diabetics also support this hypothesis (unpublished data from Dr. Sandeep Mathur lab). As pioglitazone is known to decrease insulin resistance by enhancing the process of adipogenesis, therefore its higher glycemic response in Asian Indians could possibly be because of selectively ameliorates this pathophysiologic defect.

There was wide inter-individual variation in glycemic response (coefficient of variance was 66.44%, 95.75%, and 75.22% respectively for FPG, PPG and HbA1c). Eighteen (20.45%) patients were primary non-responders in whom less than 10% decrease in HbA1c level was observed. While 70 (79.55%) patients were responders who showed more than 10% decrease in HbA1c from baseline levels after the treatment. On multiple regression analysis baseline levels of HOMA-β, HbA1c, and FPG were found to be a predictor of achieving optimal glycemic control. These findings are in line with another study where higher baseline HbA1c levels were found to be associated with optimal improvement in HbA1c levels with pioglitazone [17]. We found that glycemic control was not age-dependent, which was supported by one systematic review [18]. Only borderline statistically significant inverse relationship was observed between glycemic response and W: H ratio, HOMA-R. These findings suggest that both unimpaired beta cell function and high insulin resistance is predictor of better glycemic response to pioglitazone. Our results are consistent with those of Igarashi et al. [19]. They have reported that patients with high levels of body mass index (BMI) and homeostatic model assessment of insulin resistance (HOMA-R) at baseline were likely to respond to pioglitazone [19]. The another important implication of findings of all these studies is that the degree of glycemic response among responders is related to underlying pathophysiology of the patient instead pharmacokinetics of this drug.

The degree of response to pioglitazone among responders needs to be distinguished from primary unresponsiveness to this drug. The cause of primary pioglitazone failure is not known and is an interesting field of investigation. The present study revealed that primary non-responders to pioglitazone could not be identified on the basis of age, sex, body weight, BMI, fat distribution, insulin resistance measured as HOMA-R. Primary non-responders had higher HOMA-β and HbA1c. These findings suggest that primary non-responsiveness is neither due to beta cell exhaustion nor it is related to insulin resistance. In other words the primary unresponsiveness to pioglitazone is independent of underlying pathophysiology of diabetes.

Significant increase in body weight and BMI was noticed among responders but not in the non-responders group, which is supported by another study [20] that showed, patients receiving rosiglitazone gained substantially more weight during the study follow-up period than patients receiving glyburide or metformin. The primary non-responders also did not show any significant change in lipid parameters. In other words primary un-responsiveness to pioglitazone was

not only restricted to glycemic response, but also included lipid and weight effects of pioglitazone. As all of these effects of pioglitazone are caused by PPAR-γ receptor activation, therefore the primary un-responsiveness to pioglitazone could be due to non-activation of PPAR-γ receptor by this drug. Hence, the pharmacogenomics studies for primary unresponsiveness to pioglitazone should be designed with this fact in background.

The cause of primary failure could be various; but as discussed above, one of these reason being due to polymorphism of PPAR- γ and its co-activator gene. A large number of PPAR- γ gene polymorphs have been identified with a spectrum of phenotypic manifestations and varying frequency in different races [21]. Pro12Ala polymorph of PPAR- has been found to be associated with better glycemic response with rosiglitazone, but not with pioglitazone [22]. This polymorph is rare in Asian Indian population [7]. Two haplotype block polymorphs were found to be associated with glycemic response to another TZD, troglitazone [23].

Therefore, in the present study it was hypothesized to conduct study of PPAR- γ gene and its co-activator polymorphs for the purpose of identifying markers of primary pioglitazone failure. We designed 10 primer sets flanking variants SNP markers spanning PPAR- γ and its co-activators gene. The PCR fragments produced by amplification of these genomic regions were subjected to HRM melting analysis for screening of polymorphs of PPAR- γ and its co-activator gene.

On melting curve (HRM) analysis we found that exon 1 of PPAR-γ gene was highly polymorphic among the responders, but there was wide overlapping in Tm (melting temperature) of this fragment between responders and non-responders. Therefore, suggesting that primary unresponsiveness to this drug is not related to PPAR-γ gene. Though, a firm conclusion cannot be drawn on the basis of their HRM melting curve analysis alone. There is need of sequencing of this gene to detect variations its sequence and to further support our finding of possibly no role of polymorphs of these genes in primary unresponsiveness to pioglitazone. However one can safely conclude that the primary unresponsiveness to pioglitazone is not related to its receptor binding and the subsequent steps in the mechanism of actions of this drug. Could it be related to metabolism of this drug? Hence there is need of pharmacogenomic studies on pioglitazone focusing on the pharmacokinetics of this drug.

5. CONCLUSIONS

Pioglitazone is an effective anti-diabetic drug for Asian Indian Type 2 DM patients and decrease in HbA1c (%) levels, which is much higher as compared to what is reported for Caucasian. There was wide inter individual variations in glycemic response to pioglitazone. The glycemic response to this drug was found to be associated with baseline glycemic level and beta cell function. The present study also revealed that primary non-responsiveness to this drug could not be predicted on the basis of PPAR-Ɣ and its co-activator Gene Exon SNP markers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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