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# Differential Expression of Glucokinase activity in Indian Type-2 Diabetes Patients

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**Abstract:** Glucokinase (GK) is a type IV isoenzyme that belongs to the family of hexokinases. GK plays a role in the glucose metabolism of the liver and a glucose sensor in pancreatic β-cells involved in glucose-dependent insulin release Therefore we estimated the glucokinase levels in Indian type 2 diabetic populations. Selection of the study group was as follows: (1) Twenty nondiabetic subjects (control group), (2) thirty newly detected diabetic patients (without treatment), (3) forty three diabetic patients on combination treatment (4) thirty diabetic patients on pioglitazone alone treated. In all the subjects Anti diabetic activity criterion was taken for the diagnosis. In the same subjects fasting and post-prandial GK enzyme levels were estimated. Fasting and post-prandial Glucokinase levels in different groups were statistically significant, except between the non diabetic vs. anti diabetic drug treated group. It showed significant p-value regarding fasting and post-prandial glucokinase levels. Glucokinase levels in oral antidiabetic (combination) drugs (4.3±1.4 and 5.8±1.1 U $L^{-1}$) and pioglitazone alone treated groups showed significantly higher (3.84±0.9 and 5.1±0.7 U $L^{-1}$) than newly diagnosed type-2 diabetic patients (3.5±1.1 and 1.7±0.7 U $L^{-1}$) in fasting and post-prandial condition. Fasting and post-prandial glucokinase levels were low in newly detected, raised in drug-treated diabetic subjects and varied significantly increased in treated diabetic group. This change may be attributed to chronicity of diabetes due to effect of pioglitazone on post-prandial Glucokinase levels.

**Key words:** Glucokinase, pioglitazone, type-2 diabetes, Indian patients

## INTRODUCTION

Glucokinase (GK) is a type IV isoenzyme that belongs to the family of hexokinases (ATP: D-hexose 6-phosphotransferase; EC 2.7.1.1) and catalyzes the formation of glucose 6 phosphate in eukaryotic cells, (Dipietro *et al*., 1962; Vinuela *et al*., 1963) has a molecular mass of 52 kDa and a low affinity for glucose and is not subject to feedback inhibition by glucose 6-phosphate. The GK activity is expressed in liver (Printz *et al*., 1993), the pancreatic islets of Langerhans (Matschinsky and Ellerman, 1968), jejunal enterocytes, euroendocrine cells and brain (Jetton *et al*., 1994; Navarro *et al*., 1996; Roncero *et al*., 2000), GK plays a role as a beta cell glucose sensor by integrating blood glucose levels and glucose metabolism with insulin secretion (Matschinsky, 1990; Zelent *et al*., 2005; Matschinsky, 2000) and facilitative glucose transporter GLUT2 (Thiel *et al*., 2003) present in β-cells and hepatocytes, extracellular glucose concentrations are sensed intracellularly by GK (Richter, 1992), which determines the threshold for insulin secretion, whereas in the liver, GK facilitates hepatic glucose uptake during hyperglycemia (Cronstein, 1985). The GK has a major role in the control of blood glucose homeostasis because it is the predominant hexokinase expressed in the liver, has very high control strength on hepatic glucose disposal (Dipietro *et al*., 1962) and is the glucose sensor for insulin secretion in pancreatic Beta-cells (Agius, 2008). Glucokinase is currently considered a strong candidate target for antihyperglycemic drugs for type 2 diabetes (Matschinsky *et al*., 2006; Coghlan and Leighton, 2008; Agius, 2007). This is supported by the impact of mutations in the glucokinase gene on blood glucose concentration in humans. Inactivating mutations that lower the enzyme affinity for glucose or compromise glucokinase expression cause diabetes (maturity onset diabetes of the young type 2). Type 2 diabetes is associated with defective regulation of hepatic glucose metabolism and impaired conversion of glucose to glycogen (Basu *et al*., 2000; Krssak *et al*., 2004). This described as decreased autoregulation or glucose effectiveness (Mevorach *et al*., 1998). Hepatic glucokinase activity was shown to be either elevated in newly diagnosed type 2 diabetic patients (Van Schaftingen *et al*., 1997) or decreased in obese subjects with diabetes (Shin *et al*., 2007). Hepatic glucokinase is regulated by an inhibitory protein

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glucokinase regulatory protein (GKRP) that binds glucokinase with high affinity at basal glucose concentrations (5 mmol $L^{-1}$) and sequesters glucokinase in the nucleus in an inactive state (Agius, 2008; Payne *et al*., 2007). In the postprandial state, hyperglycemia causes dissociation of glucokinase from GKRP and translocation to the cytoplasm. It could be speculated that decreased glucose effectiveness in type 2 diabetes in humans may involve decreased glucokinase expression or impaired regulation by GKRP, as occurs in animal models of insulin resistance (O'Doherty *et al*., 1999; Postic *et al*., 2001) in addition to other metabolic defects. For hepatic glucokinase to be an effective target for antihyperglycemic drugs (GKAs) in type 2 diabetes in humans, there are must be sufficient expression of endogenous glucokinase can elicit a substantial increment in glucose phosphorylating capacity. Glucose disposal in the dysregulated diabetic state and important criterion is that glucokinase activation in diabetes that would further aggravates hepatic insulin resistance (Torres *et al*., 2009). There are no clinical data on both phenotypic or genotypic glucokinase expression and polymorphism of glucokinase in Indian type-2 diabetic patients. Therefore, we determined to study the glucokinase levels in fasting/post-prandial states in different groups of subjects.

## MATERIALS AND METHODS

Glucokinase (enzyme) from Sigma, St. Louis, MO, USA. Tris HCL, G6PD, ATP, NAD and D-Glucose were from Hi-Media Ltd., Mumbai, India. Glucose kit was from Excel Diagnostics, Hyderabad, India. Metformin and pioglitazone combination tablets were from Medibast Pharma Ltd., Chennai, India. Pioglitazone tablets were from Kare labs Pvt. Ltd., Goa.

**Study design:**

**Group 1:** Twenty non-diabetic subjects (control)
**Group 2:** Thirty newly diagnosed type 2 diabetes without treatment
**Group 3:** Forty three diabetic patients on oral antidiabetic drug treatment (metformin and Pioglitazone combination) for more than 6 months
**Group 4:** Thirty diabetic patients on oral antidiabetic drug treatment (Pioglitazone alone) for more than 6 months alone

All patients were recruited at the department of general medicine, Mahatma Gandhi memorial Hospital, Warangal andhra Pradesh, India from March 2008 to April 2009. All subjects were attending general health check up at our outpatient department (Thursday-Diabetic Care Programme) in MGM Hospital. Subjects were excluded if they had chronic gastrointestinal diseases associated with chronic pancreatitis, history of any malignant disease, history of alcohol abuse, kidney or liver failure and other diseases affecting carbohydrate metabolism. Fasting as well as post-prandial blood samples were collected from all subjects and fasting and post-prandial serum glucose and Glucokinase levels were estimated. Serum glucose levels were estimated by glucose oxidase/peroxidase (GOD/POD) method (Trinder, 1969). Different concentrations of Glucokinase (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 IU $L^{-1}$) in serum were prepared for calibration curve. Glucokinase activity was measured by a colorimetric assay (Goward, 1986). The study was approved by institutional ethics committee (Kakatiya Medical College, Warangal) and informed consent was obtained from each subject according to the principles of the declaration of Helsinki.

**Statistical analysis:** All variables are expressed as Mean±SD. Group differences of continuous variables were compared using ANOVA followed by Student-Newman Keuls post hoc test. For all analyses, a p-value<0.05 was considered to be statistically significant. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA).

## RESULTS

Glucokinase levels in fasting/postprandial states in different groups were compared (nondiabetic, newly detected and anti diabetic drug treated. Table 1 shows the clinical characteristics of all group subjects Glucokinase levels in oral antidiabetic (combination) drugs (4.3±1.4 and 5.8±1.1 U $L^{-1}$) and pioglitazone alone treated groups showed significantly higher (3.84±0.9 and 5.1±0.7 U $L^{-1}$) than newly diagnosed type-2 diabetic patients (3.5±1.1 and 1.7±0.7 U $L^{-1}$) in fasting condition. Statistical significant levels of Glucokinase in different groups represented in Table 2. Ratio of fasting/post-prandial Glucokinase is <1 in normal controls and newly detected diabetics without treatment, but the same ratio is noted >1 in chronic drug-treated group The results of present study indicate that fasting Glucokinase levels in different groups were statistically significant ($p<0.05$). Similarly post-prandial Glucokinase levels in different groups were statistically significant ($p<0.05$), except between the non-diabetic vs. anti-diabetic drug treated group.

Table 1: Clinical characteristics of subjects participating in the study

| Parameter | Non diabetic | Newly-detected | Oral antidiabetic (combination) | Pioglitazone treated |
|---|---|---|---|---|
| Age (years) | 25.85±2.4 | 48.12±12.1 | 49.9±10.6 | 48.12±12.1 |
| Sex (M/F) | 20(12/08) | 30(18/12) | 43(25/18) | 30(18/12) |
| BMI (kg $m^{-2}$) | 21.5±2.3 | 26.3±3.3 | 24.4±2.3 | 26.3±3.3 |
| Fasting glucose (mg %) | 76.8±11.4 | 169.3±23.4 | 95.1±25.4 | 107.8±18.5 |
| Post-prandial glucose (mg %) | 104.5±11.1 | 243.6±29.5 | 124.6±20.5 | 131.3±22.7 |
| Fasting glucokinase (U $L^{-1}$) | 4.6±1.00 | 3.5±1.1 | 4.3±1.4 | 3.8±0.9 |
| post prandial glucokinase (U $L^{-1}$) | 6.2±1.6 | 1.72±0.7 | 5.8±1.1 | 5.1±0.7 |

Table 2: Statistical significance levels of GK activity in different groups

| | p-value | |
|---|---|---|
| Glucokinase activity | Fasting | Postprandial |
| Non-diabetic vs. newly detected | <0.001 | <0.005 |
| Non-diabetic vs. anti-diabetic (combination) treated | <0.005 | <0.021 |
| Anti-diabetic (combination) treated vs. newly detected | <0.001 | <0.001 |
| Pioglitazone treated vs. newly detected | <0.005 | <0.052 |
| Pioglitazone treated vs. non diabetics | <0.001 | NS |
| Pioglitazone treated vs. anti-diabetic (combination) | NS | NS |

Values were expressed as Mean±SD. The p-value less than 0.05 are considered as statistically significant. NS: Non significant

## DISCUSSION

In comparison to non-diabetic group (4.6±1.0) fasting Glucokinase levels in newly detected diabetics (3.5±1.1) are significantly decreased and moderately raised in combination and pioglitazone treated (4.3±1.3 and 3.8±1.0 U $L^{-1}$). Regarding post-prandial Glucokinase, significantly decreased in newly detected group (1.7±0.22) but much elevated in combination treated and pioglitazone (5.8±0.8 and 5.1±0.7 U $L^{-1}$). Table 2 shows fasting Glucokinase levels were statistically increased in all the groups. Postprandial Glucokinase levels were also significantly decreased in all the groups except non-diabetic vs combination treated and pioglitazone but when compared to normal and treated uncontrolled group, significant p values were observed in treated group. One interesting observation is that, post-prandial Glucokinase levels were decreased in comparison to fasting Glucokinase levels, in newly detected group but not decreased. The thiazolidinediones, synthetic ligands of Peroxisomal Proliferator-Activated Receptor-ganıma (PPAR-ganıma), improve peripheral insulin sensitivity and glucose-stimulated insulin secretion in pancreatic beta-cells. To explore the role of PPAR-ganıma in glucose sensing of beta-cells, we have dissected the beta-cell-specific glucokinase (GK) promoter, which constitutes glucose-sensing apparatus in pancreatic beta-cells and identified a Peroxisomal Proliferator Response Element (PPRE) in the promoter (Kim *et al*., 2002) and other reports are thiazolidinediones (TZDs), synthetic ligands of Peroxisome Proliferator-Activated Receptor (PPAR)-γ, are known to decrease hepatic glucose production and increase glycogen synthesis in diabetic animals. Recently it was reported that glucokinase (GK) expression was increased by TZDs in the liver of diabetic ZDF rats (Kim *et al*., 2004). The concept of combination therapy for type 2 diabetes has been widely explored with drugs that target different organs such as insulin secretagogues and insulin sensitizers or metformin. The combined effects of up regulation of glucokinase and down regulation of phosphorylase-a on hepatocyte glycogen metabolism because both enzymes have a high control strength on glycogen metabolism (Agius *et al*., 1996; Aiston *et al*., 2001) and both glucokinase activators (Grimsby *et al*., 2003). In animal studies, it has been shown that chronic hyperglycemia decreases glucokinase activity and that restoration of euglycemia results in the normalization of Glucokinase activity (Nawano *et al*., 2000). Nonetheless, the fractional inactivation of phosphorylase is modest compared with the increase in glycogen synthesis. Stimulation of glucokinase translocation by insulin (Agius and Peak, 1993), these observations may be due to chronicity of diabetes mellitus or with pioglitazone treatment or uncontrolled metabolic status in spite of Glucokinase being high. Low levels of Glucokinase (fasting and post-prandial) in early stage of Diabetic Mellitus (newly detected) show a reactive phase in the evolution of diabetes mellitus. This reactive phase is whether due to decreased in the early onset of diabetes is unknown.

## CONCLUSIONS

Indian ethnic groups show decreased post-prandial response of Glucokinase in newly detected diabetic group without treatment. Fasting Glucokinase levels show negative correlation with duration of diabetes. But post-prandial Glucokinase levels are significantly lower in newly detected group when compared to normal controls. This is a contradictory finding to the conclusion. The present study results indicates that the serum Glucokinase activity in patients with type-2 diabetes mellitus correlates negativity with blood glucose levels, but is not acutely affected by food intake. This special reactive phase is seen in Indian ethnic groups. Further studies are required in this line. Reversal of fasting/post-

randial Glucokinase ratio in the course of diabetes mellitus can be taken as a parameter for therapeutic intervention either by multiple drugs or insulin. This reversal of ratio might also be revealing failure of other response.

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