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α-amylase Expressions in Indian Type-2 Diabetic Patients

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α-amylases are enzymes which hydrolyze starch molecules to give diverse products including dextrins and progressively smaller polymers composed of glucose units which causes hyperglycemia and development of type 2 diabetes mellitus. Therefore, we estimated the α-amylase activity, HbA1C and blood glucose in Indian type 2 diabetic patients. Study group's follows: 20 Healthy subjects, 30 newly detected patients and 43 type 2 diabetics on treatment with metformin and pioglitazone combination and 30 pioglitazone alone treated. In all subjects blood sugar was estimated and anti diabetic activity criterion was taken for the diagnosis. In the same subjects fasting and post-prandial α-amylase activity was estimated, HbA1c levels were compared with α-amylase activity in healthy and treated subjects. The α-amylase activity in type 2 diabetic patients was significantly different from control subjects and significant relation between α-amylase activity and HbA1c was observed in diabetic subjects ($p<0.01$), in type 2 diabetic patients with HbA1c >12% showed significantly ($p<0.01$) higher α-amylase activity than newly diagnosed and healthy, for three months treatment in type 2 diabetic patients showed a significant controlled the blood glucose, elevated alpha amylase levels. Post-prandial α-amylase activity was markedly raised in type 2 diabetes, this could contribute to the increased in circulating blood glucose. Fasting and post-prandial α-amylase activity and lipid profiles were significantly controlled in pioglitazone alone and pioglitazone with metformin combination treated groups. This change may effect of metformin and pioglitazone on post-prandial α-amylase activity.

Key words: α-amylase, blood glucose, HbA1C, type 2 diabetes

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INTRODUCTION

Diabete mellitus (DM) is a group of disorders of carbohydrate metabolism characterized by hyperglycemia, with an estimated 170 million patients worldwide in the year 2000. Its prevalence is rapidly increasing (Wild *et al*., 2004). It is a clinically complex and associated with many serious complications including kidney failure, blindness and cardiovascular disease (Baliga and Weinberger, 2006). The chronic Diabetes mellitus details a substantial decrease in quality of life and life expectancy (Coffey *et al*., 2002; Stephens *et al*., 2006). In the previous studies, diabetes is increased with metabolic disorders of carbohydrates (Ben-Aryeh *et al*., 1988; Streckfus *et al*., 1994; Belazi *et al*., 1998). In recent studies pancreatic dysfunction may leads to diabetes mellitus (Hardt *et al*., 2000; Lernmark, 2000).

Clinical investigation observations indicate close relation between pancreatic exo- and endocrine parts (Okabayashi *et al*., 1988; Aughsteen and Kataoka, 1993; Meral *et al*., 2001). Insulin dysfunction and glucagon activation is due to pancreatic impairment (Adeghate, 1999; Taniyama *et al*., 1999; Hardt *et al*., 2000; Lernmark, 2000; Harding *et al*., 2001). The decreased production of exocrine secretion also influence on insulin deficiency (Okabayashi *et al*., 1988; Aughsteen and Kataoka, 1993; Kim *et al*., 2000).

Interestingly, the relation between insulin and glucagon concentrations in extracellular fluid and their antagonistic actions also seem to play a key role in pancreatic exocrine function disorders. Insulin secretion is enhancing the exocrine activity like alpha amylase functions but glucagon inhibits it (Taniyama *et al*., 1999; Hardt *et al*., 2000). Amylases are enzymes that hydrolyze the carbohydrates into small glucose particles (Windish and Mhatre, 1965).

All the alpha amylase enzyme molecules acts on alpha 1-4, 1-6 links of glucose residues (Van der Maarel *et al*., 2002). Amylases were categorized into two, that one is endoamylase and another one is exomylase the endoamylases are involved in hydrolysis of carbohydrates in to different chain lengths of linear and branched molecules and exoamylases reduced in to short molecules (Gupta *et al*., 2003). The enzyme alpha amylase is one of the most important in human body responsible of hydrolysis of starch in to small sugar molecules (Alexander, 1992).

Amylase is one of the main enzymes produced in exocrine pancreatic cells, may be recognized as an adequate indicator of organ's activity both in physiological and pathological states. Moreover, these studies might help in the development of effective alpha amylase inhibitors.

MATERIALS AND METHODS

Amylase kit purchased from Becan Pvt. Ltd, Mumbai, India. Glucose kit was from Excel Diagnostics, Hyderabad, India. Metformin and pioglitazone combination tablets were from Sun Pharma Pvt Ltd, Sikkim, India. Pioglitazone tablets were from Sun Pharma Pvt Ltd, Sikkim.

Study design follows:

Group 1: Twenty non-diabetic subjects (control)
Group 2: Thirty newly diagnosed type 2 diabetes without treatment
Group 3: Thirty newly diagnosed type 2 diabetic patients with pioglitazone treatment for three months
Group 4: Forty three diabetic patients on oral antidiabetic combination treatment for three months (pioglitazone with metformin)

All patients were recruited at the department of general medicine, Mahatma Gandhi memorial Hospital, Warangal andhra Pradesh, India, from March 2008 to April 2010. All subjects were attending general health check up at our outpatient department 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 (Trinder, 1969), alpha amylase levels and fasting lipid profiles (Bucolo and David, 1973) were estimated by a colorimetric assay. The study was approved by institutional Human ethics committee (University College of pharmaceutical sciences, Kakatiya University, 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 $p<0.05$ was considered to be statistically significant. All analyses were performed using INSTAT 1.12 (Graph-Pad Software, Inc., San Diego, CA).

RESULTS

Alpha amylase levels in fasting and postprandial statuses in different groups were compared (nondiabetic,

Table 1: Clinical characteristics and alpha amylase expression in participating subjects in this study

Parameter	Non diabetic	Newly-detected	Pioglitazone treated	Oral antidiabetic (combination)
Age (years)	25.85±2.4	44.12±12.1	44.12±12.1	49.9±10.6
Sex (M/F)	20 (12/08)	30 (17/13)	30 (17/13)	43 (25/18)
BMI (Kg m^{-2})	21.5±2.3	26.3±3.3	24.3±3.3	23.5±2.3
Fasting glucose mg (%)	76.8±11.4	169.3±23.4	107.8±18.5**	95.1±25.4***
Post-prandial glucose mg (%)	104.5±11.1	243.6±29.5	131.3±22.7***	124.6±20.5***
Fasting α-amylase activity (U L^{-1})	14.2±1.1	55.32±3.9	33.1±2.9*	35.6±1.7*
Post-prandial α-amylase activity (U L^{-1})	33.1±1.4	81.2±4.2	40.4±1.9***	37.2±1.5***
HbA1c (%)	4.7±0.31%	14.3±1.50%	7.3±1.11% ***	5.8±0.56%****

Data values were expressed as Mean±SD. p-value less than 0.05 are considered as statistically significant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

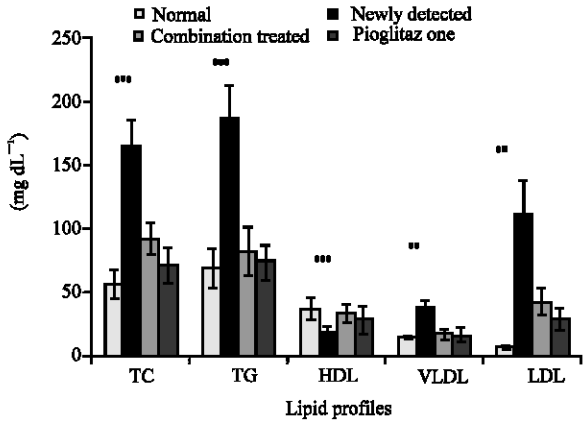

Fig. 1: Serum lipid profiles in healthy, newly detected and treated groups, Data values are expressed as Mean±SD. ** p<0.01, *** p<0.001

newly detected, pioglitazone alone treated and combination drug treated). Table 1 represents the clinical characteristics of all group subjects alpha amylase levels fasting and postprandial in oral antidiabetic (combination) drugs (35.6±1.7 and 37.2±1.5 U L^{-1}) and pioglitazone alone treated groups showed significantly lowered (33.1±2.9 and 40.4±1.9 U L^{-1}) than newly diagnosed type-2 diabetic patients (55.32±3.9 and 81.2±4.2 U L^{-1}). Statistical significant levels of alpha amylase in different groups represented in Table 1.

The results of present study indicates that fasting alpha amylase levels in different groups were statistically significant ($p<0.05$). The significant relation between alpha amylase and HbA1c was observed in type 2 diabetic patients ($p<0.001$). Type 2 diabetes with HbA1c >12% showed significantly ($p<0.031$) higher alpha amylase activity (Mean±SD; 74.3±4.6 U L^{-1}) and lipid profiles LDL, Triglycerides and total cholesterol levels (Fig. 1) than healthy ((HbA1c<7% and alpha amylase activity (23.4±1.2 U L^{-1}, respectively)). Variations in alpha amylase activity for 3 months treatment in type 2 diabetic patients showed a significant reduced the HbA1c ($p<0.001$), similarly fasting and post-prandial, alpha amylase levels and LDL, Triglycerides and total cholesterol levels in pioglitazone and combination treated groups but increase the HDL levels ($p<0.001$), except between the non-diabetic Vs anti-diabetic drug treated groups.

DISCUSSION

The results shows that in the case of type 2 diabetes their FBS and HbA1c levels were far above the normal range but their serum alpha amylase enzyme levels were significantly elevated. By Pioglitazone monotherapy their FBS, HbA1c and alpha amylase levels were well controlled to the near normal values. The pioglitazone therapy is very effective for obese type 2 diabetic patients. The group 4 patients showed a marked amelioration of hyperglycemia and alpha amylase and HbA1c with combination therapy of pioglitazone and metformin. The highest fall of alpha amylase observed in combined therapy may be due to two factors. i.e., due to very high pretreatment level of FBS and also due to insulin sensitizer action of pioglitazone. Similarly the comparatively higher FBS fall for pioglitazone group is the drug of choice that worked here also as it enhances the peripheral uptake of glucose. We can observe a parallel decrease of HbA1c with alpha amylase in the pioglitazone and metformin combined therapy group. Therefore, combination therapy is more effective than monotherapy for controlling.

Metformin Inhibits Proinflammatory Responses and Nuclear Factor-B in Human Vascular Wall Cells and antioxidant activity (Winder and Hardie, 1999; Isoda *et al*., 2006). Several reports have demonstrated an anti-inflammatory action of the specific ligands of PPARγ activators of PPARγ inhibit the generation of pro-inflammatory cytokines such as interleukin-1β (IL- 1β), tumor necrosis factor-a (TNF-a) and interleukin-6 (Suganami *et al*., 2005). PPAR γ agonists increases HDL particles to inhibit dylipidemea and antioxidant activity (Barter *et al*., 2004). Pioglitazone reduced serum triglycerides and increased HDL-C to a greater extent than either rioglitazone or rosiglitazone. In addition, trioglitazone and rosiglitazone increased LDL-C whereas, pioglitazone did not (Van der Maarel *et al*., 2002;

Gupta *et al*., 2003). All these studies and our present findings stress the importance of pioglitazone in combination therapy but with all the required precautions.

CONCLUSIONS

Indian ethnic group's shows increased Post-prandial Alpha Amylase levels were significantly higher in type 2 diabetic group when compared to normal controls. This is a contradictory finding that serum Alpha Amylase activity in patients with type-2 diabetes mellitus relatively positive with HbA1c, blood glucose and lipid profile levels were completely controlled to normal by pioglitazon alone and combination with metformin treatment but increased the HDL levels. The results may provide important information concerning the activity of alpha amylase in type 2 diabetic patients and its association with alpha-amylase and glucose.

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