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Original Article

Biochemistry Section

Evaluation of Biochemical Markers Serum Amylase and Serum Lipase for the Assessment of Pancreatic Exocrine Function in Diabetes Mellitus

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ABSTRACT

Introduction: Diabetes mellitus (DM), a metabolic disorder characterized by hyperglycaemia, associated with deficiency or resistance to insulin indicates endocrinal abnormality of the pancreas. Amylase and lipase are enzymes secreted by the exocrine portion of the pancreas. Endocrinal derangement observed in diabetes may interfere with the exocrine function of the pancreas.

Aim: To estimate the levels of fasting blood sugar, serum lipase, serum amylase in patients of type 1 and type 2 DM. Than comparing them with healthy controls and to study the effect of type 1 and type 2 DM on pancreatic exocrine function using serum levels of amylase and lipase as biochemical marker.

Materials and Methods: This study was conducted at GMERS Medical College and Hospital from Dec 2015 to July 2016. Thirty patients of type 1 DM and 30 patients of type 2 DM, who were already diagnosed and taking treatment, were included in this study. A total number of 30 apparently healthy individuals were recruited as the control group in our study. Fasting venous blood samples were collected from the cases as well as the controls and they were analysed by using semi auto analyser for blood glucose, serum amylase and serum lipase. The results were analysed statistically by using SPSS software. Values were expressed as means ± SD.

Results: We found statistically significant ($p<0.01$) low values for serum amylase and serum lipase in patients with type 1 and type 2 DM as compared to healthy controls. Fasting blood sugar was significantly higher in cases as compared to controls. We found negative correlation of fasting blood sugar level with serum amylase and serum lipase and positive correlation of serum amylase with serum lipase in both type 1 and type 2 DM.

Conclusion: Our study clearly demonstrated that in type 1 and type 2 DM, there was increase in fasting blood sugar with decrease in serum amylase and serum lipase which signifies the derangement of endocrine-exocrine axis of the pancreas. Serum amylase and serum lipase can be used as biochemical markers for assessment of pancreatic exocrine function.

Keywords: Endocrine- exocrine axis, Fasting blood sugar, Insulin resistance, Pancreatic enzymes, Type 1 and type 2 diabetes mellitus

INTRODUCTION

The prevalence of diabetes mellitus (DM) is developing rapidly and expected to double globally from 171 million in 2000 to 366 million in 2030 with a maximum rise in India. It is expected that DM may affect up to 79.4 million individuals in India by 2030 [1]. At present India is the capital of DM disease in the world [2].

Biochemical cause of DM in type 1 and type 2 is decreased or lack of insulin secretion and resistance to insulin action respectively. Insulin resistance is defined as decreased sensitivity of target organ to the biochemical effects of insulin [3]. Insulin is secreted by islets cell of the pancreas. Pancreas is both an endocrine and an exocrine gland with clusters of endocrinal islet cells dispersed among exocrinal acinar cells [4].

Anatomical structure of pancreas is made up of 84% exocrine component, 2% endocrine part, 10% extracellular matrix and 4% ductal cells and blood vessels. Acinar cells and islet cells are in close proximity with each other. Defect in islet cells observed in diabetes may disturb neighbouring acinar cells of the pancreas [5]. Defects in insulin secretion and function leads to hyperglycaemia and may affect the enzyme synthesis and release from exocrine pancreas. Enzymes which are released from pancreas are amylase, lipase and proteases [6].

Several clinical research studies have shown that low serum amylase was associated with diffuse pancreatic destruction because of advanced pancreatic diseases, such as chronic pancreatitis [7,8]. Recently some studies showed that low serum amylase values are associated with metabolic syndrome and diabetes [9,10]. High value of amylase and lipase enzyme is seen in pancreatitis, pancreatic cancer and pancreatic duct obstruction [11].

Even though exocrine-endocrine relationship in the pancreas has been a center of attention in animal and cellular studies, in the human diabetic research, very little concern on pancreatic exocrine function has been paid. Most of the studies are targeted on metabolic derangement induced by persistent hyperglycaemia due to decreased insulin levels. There are number of studies regarding high values of serum amylase and lipase in acute pancreatitis [12,13] but very few studies are seen regarding significance of low serum amylase and lipase levels in clinical condition like DM.

Animal and cellular studies showing the relationship between the endocrine and the exocrine pancreas have persistently observed that insulin affects amylase secretion via islet acinar cell axis. Insulin binds with its receptor on acinar cells and stimulates amylase secretion through number of ways [14]. There are confusing results regarding serum lipase levels in DM.

We found very few studies in India regarding serum amylase, serum lipase and its association with DM. So the present study was conducted to estimate serum amylase along with serum

lipase in patients of type 1 and type 2 DM and to assess the role of amylase and lipase as a biochemical markers for pancreatic exocrine function.

MATERIALS AND METHODS

The study was conducted in clinical Biochemistry Department of GMERS Medical College and Hospital, Valsad, Gujarat, India.

An excel based software was used to calculate the sample size.

The following formula was used to estimate sample size.

n= [(Z1+Z2)/Cr)] ^2

Where Cr = ½ {LOGe (1+r)/(1-r)}

Correlation coefficient = 0.712 (as estimated in pilot study).

Cr (Fisher's Arctanh transformation) = 0.891

Level of confidence = 99%, Z1 (Z-value associated with alpha) = 2.57

Power of test = 90%, Z2 (Z-value associated with beta) = 1.28)

Putting these value in the above formula we got sample size (n) = 19

It was a case control study of 90 subjects divided into three groups including 30 apparently healthy controls, 30 cases of type 1 DM and 30 cases of type 2 DM. All the participants gave written informed consent and this study design was approved by the institutional research and ethics committee. The study was carried out for a period of 8 months from December 2015 to July 2016. Exclusion criteria's for both cases and controls were patients with history of hypertension, hypercholesterolemia, cardiovascular disease, hepatic disorders, chronic renal insufficiency and alcohol abuse. Fasting blood samples were collected after overnight fast by venipuncture in plain vaccutainer and sodium fluoride bulb. Grossly haemolysed and lipemic samples were excluded. All the samples were analysed by Microlab RX 50 diagnostic equipment. The estimation of Fasting Blood Sugar (FBS) was done by the glucose oxidase-peroxidase method on the blood samples collected in sodium fluoride bulb. The amylase was estimated by a colorimetric enzymatic method (CNPG2 method). Serum lipase was measured by advanced homogenous micelle technology method.

STATISTICAL ANALYSIS

The data was analysed by SPSS (Statistical Package for the Social sciences) software version 17. Data was analysed by using Analysis Of Variance (ANOVA) and Tukey's post-hoc test was applied for the comparison of variables in all three groups. Pearson's correlation co-efficient (r) were calculated to study the correlation of variables in type 1 and type 2 DM. The p-value <0.05 was considered as statistically significant.

RESULTS

The study was conducted on 30 normal healthy volunteers (18 males, 12 females) with average age of 35.73 ± 4.53years, 30 type 1 diabetic patients (17 males, 13 females) with average age of 30.37 ± 3.92years and 30 type 2 diabetic patients (15 males, 15 females) with average age of 54.33 ± 3.50years.

[Table/Fig-1] shows mean± SD values, F-ratio and p-values of fasting blood sugar, serum amylase and serum lipase in controls, type 1 DM and type 2 DM patients.

[Table/Fig-1a] shows that FBS level was significantly increased in type 1 and type 2 diabetes patients as compared to control group. It also shows that FBS level was significantly elevated in type 1 DM as compared to type 2 DM.

[Table/Fig-1b] shows that serum amylase was significantly decreased in type 1 and type 2 diabetes as compared to control group. Decrease in serum amylase was statistically more significant in type 1 DM as compared to type 2 DM.

[Table/Fig-1c] shows that that serum lipase was significantly decreased in type 1 diabetes as compared to control group. Decrease in serum lipase was also statistically significant in type 1 DM as compared to type 2 DM. Decrease in serum lipase in type 2 DM was statistically not significant as compared to controls.

[Table/Fig-2] shows correlation of variables in type 1 DM. There was negative correlation of FBS with amylase and lipase. Correlation between serum amylase and serum lipase in type 1 DM was positive.

[Table/Fig-3] shows that there was negative correlation of FBS with serum amylase and lipase in type 2 DM. It was also observed that decreased values of serum amylase were associated with reduced levels of serum lipase in type 2 diabetes patients.

Variable	Control (n= 30)	Type 1 DM (n= 30)	Type 2 DM (n= 30)	'F' Ratio	p-value	Signi-ficance
FBS (mg/dl)	89.83 ± 8.72	212.93 ± 51.96	172.33 ± 32.59	92.28	<0.001	Signi-ficant
Sr. amylase (u/L)	80.40 ± 20.26	32.33 ± 19.99	56.37 ± 23.43	37.78	<0.001	Signi-ficant
Sr. lipase (u/L)	38.67 ± 12.42	18.90 ± 10.3	26.13 ± 13.23	20.67	<0.001	Signi-ficant

[Table/Fig-1]: Comparison of mean of variables in all three groups.

Sr. No.	Comparision of Mean FBS	p-value	Significance
1.	Control Vs Type 1 DM	<0.001	Significant
2.	Control Vs Type 2 DM	<0.001	Significant
3.	Type 1 DM Vs Type 2 DM	<0.001	Significant

[Table/Fig-1(a)]: Comparison of mean FBS in all three groups with tukey's post-hoc test.

Sr. No.	Comparision of Mean Serum Amylase	p-value	Significance
1.	Control Vs Type 1 DM	<0.001	Significant
2.	Control Vs Type 2 DM	<0.001	Significant
3.	Type 1 DM Vs Type 2 DM	<0.001	Significant

[Table/Fig-1(b)]: Comparison of mean serum amylase in all three groups with tukey's post-hoc test.

Sr. No.	Comparision of Mean Serum Lipase	p-value	Significance
1.	Control Vs Type 1 DM	<0.001	Significant
2.	Control Vs Type 2 DM	0.06	Not Significant
3.	Type 1 DM Vs Type 2 DM	<0.001	Significant

[Table/Fig-1(c)]: Comparison of mean serum lipase in all three groups with tukey's post-hoc test.

Sr. No.	Correlation between	Pearson's correlation co-efficient (r)	p-value	Significance
1.	FBS and Serum Amylase	-0.734*	<0.001	Significant
2.	FBS and Serum Lipase	-0.891*	<0.001	Significant
3.	Serum amylase and Serum lipase	0.78**	<0.001	Significant

[Table/Fig-2]: Correlation of variables in Type 1 Diabetes patients.
* Negative Correlation; ** Positive correlation

Sr. No.	Correlation between	Pearson's correlation co-efficient (r)	p-value	Significance
1.	FBS and Serum Amylase	-0.895*	<0.001	Significant
2.	FBS and Serum Lipase	-0.803*	<0.001	Significant
3.	Serum amylase and Serum lipase	0.716**	<0.001	Significant

[Table/Fig-3]: Correlation of variables in Type 2 Diabetes patients.
* Negative Correlation; ** Positive correlation

DISCUSSION

There are many animal studies and very few human studies which tried to probe the biochemical features and the underlying mechanisms to link the endocrine islet cells and the exocrine acinar

cells. Accordingly we also studied serum amylase, serum lipase and fasting blood sugar to correlate islet and acinar cell linkage. We found significantly low amylase and lipase level in type 1 DM and significantly low amylase level in type 2 diabetic groups as compared to that of healthy controls. We also found significantly higher FBS in type 1 and type 2 DM as compared to controls. Similar results were found by Aughsteen A et al., who observed decreased values of serum amylase 20.4±8.8u/l and lipase 19.8±5.6u/l in type 1 DM and reduced amylase 35.3±17.8u/l and lipase 26.5±7.8u/l in type 2 DM [10]. Kei Nakajima, Swislocki A et al., Snehankar K et al., also observed decreased serum amylase in DM [15-17].

In one study, we found streptozotocin-diabetic rats showed a reduction of 66% in amylase and 43% in lipase in pancreatic tissue homogenates. The amylase and lipase levels were returned nearly to control values after in-vivo insulin administration [18].

The study of Skrha J et al., on insulin dependent diabetic patients demonstrated a lowered serum lipase and isoamylase levels. This observation may be due to decreased acinar cell function in the vicinity of insulin depleted islets [19]. Similarly reduction in the other pancreatic enzymes like elastase, trypsin and chymotrypsin has been reported by other studies [20,21]. Analysis of pure pancreatic secretions aspirated from the pancreatic duct demonstrated a significant decrease in amylase level with minimal changes in bicarbonate and lipase concentrations in diabetic patients with uncontrolled hyperglycaemia [22].

However, in contrast to our results, Hattf BF et al., found significant increase in serum amylase level in diabetic group (175.35 ± 21.74 u/l) as compared to the control group (40.19 ± 10.50u/l) [23]. Few other studies also recorded conflicting results regarding amylase and lipase in diabetes presented with ketoacidosis [24-26].

It was observed that the hormones like insulin and glucagon secreted by pancreas, influence the enzyme synthesis and its release from the exocrine pancreas. Insulin has a trophic/stimulatory effect on the acinar cells, whereas glucagon has an inhibitory influence on the exocrine secretions. Which leads to decrease in the sensitivity of the diabetic pancreatic acini to secretagogues. So, the deficiency of insulin and the excess of glucagon in diabetes affect the internal milieu of the pancreas. It leads to decrease in the total volume, the amylase secretions and the lipase content of its exocrine secretions [5,27].

Similarly diabetic neuropathy may lead to impaired entero-pancreatic reflexes and exocrine dysfunction [28]. Few studies in recent time observed that cytokines such as TNF-alpha (tumour necrosis factor), TGF-alpha, TGF-beta 1 (transforming growth factors), gastrin and low regulatory gene functions may interact and impair the exocrine and endocrine functions [29-31].

Patel R et al., in their study explained that reduced amylase secretion in the diabetic pancreas may be due to reduced cytosolic free calcium concentration (Ca_2^+) and gene expression for amylase and not to the gene expression of cholecystokinin (CCK) a receptor in pancreatic acinar cells [32].

We observed that the low serum amylase and lipase levels in diabetes were associated with increased blood glucose level (negative correlation) due to impaired insulin action either because of insulin resistance and/or inadequate insulin secretion. Decrease in amylase level associated with decreased level of lipase in both type 1 and type 2 DM (positive correlation) was due to insufficiency of pancreatic exocrine acinar cells. Majority of the diabetics have been found to have pancreatic fibrosis and other findings such as atrophy, fatty infiltration and loss of the exocrine acinar cells.

LIMITATION

Age and sex matching of controls could not be possible with type 1 and type 2 DM due to occurrence of disease in different age groups.

Facility for the HbA1C estimation (instrument) is not available in our set up that's why we did not include HbA1C in our study.

CONCLUSION

Our study demonstrated that serum amylase and serum lipase levels were decreased significantly in type 1 and type 2 DM. These parameters whether to be accepted as a reliable biochemical markers of pancreatic exocrine function and its insufficiency in other clinical conditions should be validated by extensive, large scale research and clinical studies. The role of Serum amylase and serum lipase as biochemical markers of pancreatic exocrine function in the treatment and progress of the DM should be investigated further in detail.

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