

ISO-AMYLASE ELECTROPHORETIC PATTERNS IN HYPERAMYLASEMIC CONDITIONS – A LEADING FOOTPRINT

ARUL SENGHOR KA*, SAROJ SAPKOTA, EBENEZER WILLIAM

Department of Biochemistry, SRM Medical College Hospital & RC, Kattankulathur, Chennai, Tamil Nadu, India.

Email: arul_senghor@yahoo.co.uk

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ABSTRACT

Objective: Enzyme amylase in humans is a digestive enzyme that acts to cleave starch into smaller carbohydrates. The iso-enzymes of amylase are salivary (S) type and pancreatic (P) type with different characteristics properties. Hyperamylasemia is a condition with increased total serum amylase activity. It is often difficult to interpret the actual defective source with a total serum amylase activity. Therefore, this study of iso-amylase patterns by electrophoretic technique aids in recognizing the correct source of origin of the enzyme amylase.

Methods: A total of 80 subjects aged ranges from 20-60 years were recruited from SRM Medical College Hospital and Research center, Tamil Nadu. The subjects included were subdivided as Group 1 of 20 acute pancreatitis, Group 2 of 20 diabetes mellitus, Group 3 of 20 cholecystitis and Group 4 of 20 normal healthy subjects. Serum samples were obtained and analyzed in autoanalyzer and subjected to agarose gel electrophoresis for electrophoretic separation of isoamylase bands.

Results: Total serum amylase activity was measured. Electrophoretic iso-enzyme patterns of serum amylase of normal healthy person showed a narrow band of S-type toward the anode, followed by the P-type from the point of application. Electrophoresis of sera of pancreatitis showed only a single prominent P-type band, cholecystitis sera showed a thin S-type with P-type band and diabetic sera had a broad S-type and thin P-type isoamylase bands.

Conclusion: Agarose gel electrophoresis is a simple technique and can be run with minimal efforts. The isoamylase band patterns observed differentiates the source of origin of serum amylase. Thereby aids the physicians to take the next right leading step and decide necessary further investigations.

Keywords: Isoamylase, Hyperamylasemia, Agarose gel electrophoresis.

INTRODUCTION

According to IUBMB system of enzyme classification, the enzyme amylase belongs to the hydrolase class. The human amylase enzyme is α -amylase. Its alternative name is 1,4- β glucanglucanhydrolase and catalyzes the hydrolysis of α -1,4 glycosidic linkage in polysaccharides to yield limit dextrin, maltose and residual glucose as end products [1]. Human amylase requires calcium as metallo-enzyme, which is required for full enzymatic activity. This calcium confers increased structural rigidity and also increased resistance to proteolysis. The optimal pH of serum amylase is 6.9-7 [4]. It has two isoenzymes, P-type and S-type derived from the closely linked loci on chromosome 1 [2,3].

Salivary amylase (S-type) secreted from the salivary gland is also called as ptyalin. It breaks large insoluble starch molecules. However, loses all its activities have it reaches the stomach because of the acidic environment. Pancreatic amylase (P-type) is the major starch hydrolyzing enzyme secreted in pancreatic juice. It shows activity in alkaline medium, thereby favors further digestion of carbohydrates.

The isoenzyme of amylase is proposed to be due to post-translational modification like deamination, glycosylation and deglycosylation example S-type isoamylase. However, P-type isoamylase can only be deaminated and not glycosylated. The anodic migration of S-type isoamylase is contributed by deamination, which results in loss of the amide group from glutamine and asparagine to generate glutamic acid and aspartic acid. It results in a change from an electrically neutral amino acid to one that is negatively charged and causes the protein to exhibit an altered and more anodic migration on electrophoresis [5].

Leach *et al.* determined the molecular weight of S-type and P-type isoamylases by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Salivary amylase electrophoretic pattern showed two

bands (Sal A and Sal B) based on differences in molecular weight that is attributed to the presence of glycosylation on Sal A and its absence in Sal B isoform [6]. The serum amylase concentration reflected the balance between the rates of amylase entry into the circulation and decreased metabolic clearance from the blood.

Hyperamylasemia is a condition with elevated serum amylase level raised from either from pancreatic or non-pancreatic origin as shown in Table 1.

Thus, separation and quantifying the iso-enzyme can be clinically valuable. Thus, our work aimed to study the electrophoretic patterns of serum amylase of normal and hyperamylasemic conditions.

METHODS

About 80 subjects aged 20-60 years were recruited for the study who was admitted in SRM Medical College Hospital and Research Centre. The subjects included were subdivided into Group 1 of 20 patients with acute pancreatitis, Group 2 of 20 patients with cholecystitis, Group 3 of 20 patients with uncontrolled diabetes mellitus and Group 4 of 20 normal healthy persons. The study was approved by the Institutional

Table 1: Hyperamylasemic conditions with difference in activity of isoamylase enzyme

Diseases	Activity of S-type isoamylase	Activity of P-type isoamylase
Pancreatitis	↓	↑
Mumps/salivary gland infection	↑	↓
Biliary tract disease	↓	↑
Diabetic ketoacidosis	↑	↓
Renal failure	↑	↑

ethical committee. Also, written consent was obtained from all the subjects under study.

Serum samples were obtained and estimated for total serum amylase activity by enzymatic photometric test in Olympus AU 400. Agarose gel electrophoresis of serum amylase was done based on the method of Royse and Jensen [7]. Electrophoresis buffer was prepared with 5.77 g of tris, 2.47 g of barbital and 9.74 g of sodium barbital dissolved in 0.9 L of distilled water and makeup to 1 L. The pH is adjusted to 8.8. The staining dye was prepared with 8 phadebas amylase tablets dissolved in 10 ml of 0.9% saline solution with 0.5% of bovine serum albumin. About 2.5 g of electrophoretic grade agarose (Bio Rad) was dissolved in 250 ml of the buffer, and the suspension was boiled with stirring. Agarose gel is prepared over the slides loaded with 20 µl of the sample and electrophoresis was run for 2 hrs 30 minutes, staining dye was poured over the gel and maintained for 2 hrs. The gel is fixed with ethanol for 1 hr. Finally, isoamylase bands are visualized.

RESULTS

Electrophoretic band patterns as shown in Fig. 1 of normal healthy persons showed two clear blue colored bands; one is S-type toward the anode, followed by P-type isoamylase band from the point of application.

Serum amylase enzyme activity is high in acute pancreatitis (566.45±94.23 IU/L) and the isoamylase band patterns showed only a single characteristic P-type isoamylase band and the S-type isoamylase band was completely absent (Fig. 2). Mean of total serum

amylase activity of uncontrolled and cholecystitis was 244.4±18.41 and 216.85±85.21 IU/L, respectively.

As shown in Fig. 3, sera of uncontrolled Type 2 diabetic subjects showed broad S-type of isoamylase band with fade and thin P-type isoamylase band. Fig. 4 in cholecystitis patients, the electrophoretic pattern had a prominent P-type isoamylase band with thin and faded S-type isoamylase band. As a comparison (Fig. 5) the sera of normal persons with hyperamylasemic conditions showed the well-differentiated isoamylase bands between the cases.

DISCUSSION

Total serum amylase activity is reported to be elevated not only in acute pancreatitis but also in many other pathological conditions. Hence, the need of separation and quantification of amylase isoenzymes to differentiate the abnormal source of origin of amylase is necessary. After agarose gel electrophoresis of normal sera, we found two blue colored band patterns P-type and S-type. In sera of normal humans the amount of salivary and the pancreatic isoamylase activity is equal thereby, we observed the bands of equal bandwidth and it resembled the pattern described by Gillard et al. [8].

S-type isoamylase band had an anodic migration than the P-type isoamylase band. This is due to the reason that S-type is more negatively charged than P-type at the pH of 8.8. Furthermore, Lebenthal suggested that the molecular weight of S-type isoamylase is 55,000 Da whereas; P-type isoamylase is 62,000 Da which causes the fast migration of S-type isoamylase during electrophoresis [9].

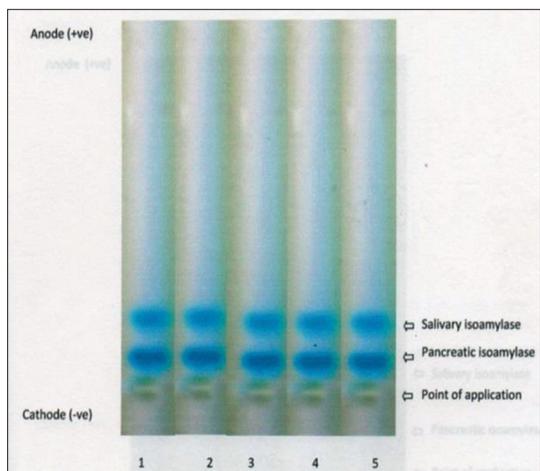


Fig. 1: Agarose gel electrophoretic pattern of normal sera

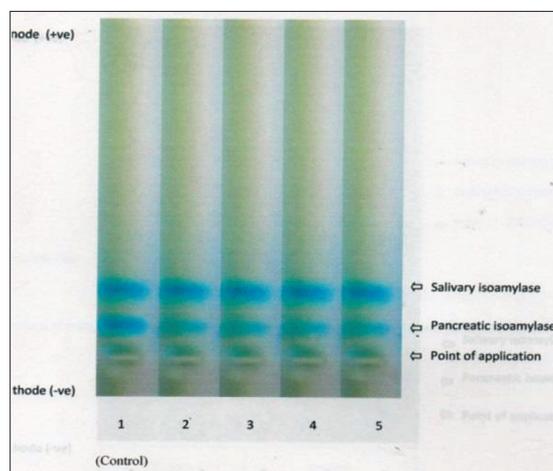


Fig. 3: Agarose gel electrophoretic pattern of diabetic sera

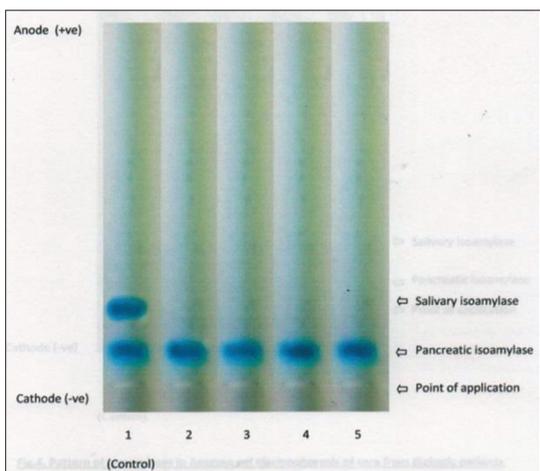


Fig. 2: Agarose gel electrophoretic pattern of acute pancreatitis sera

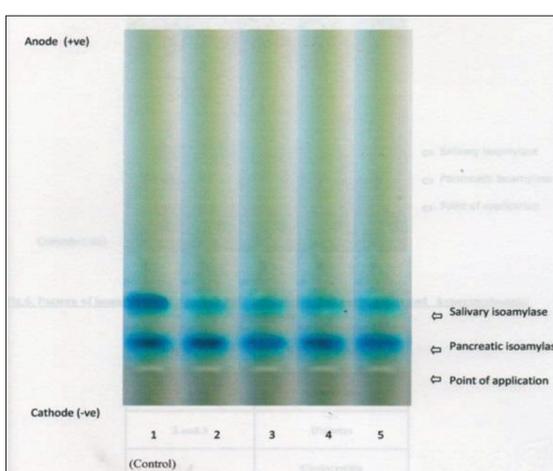


Fig. 4: Agarose gel electrophoretic pattern of cholecystic sera

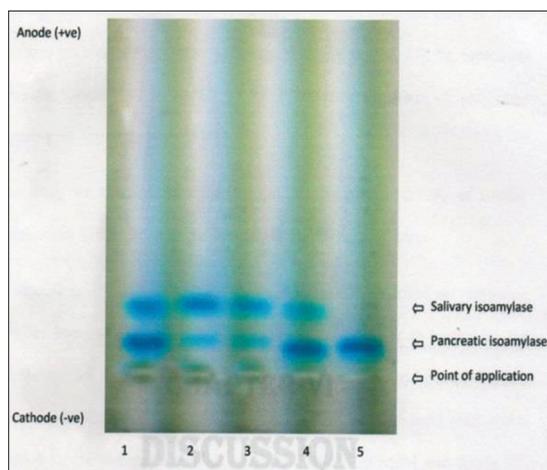


Fig. 5: Agarose gel electrophoretic patterns of hyper amylasemic conditions. Well 1 (normal sera) well 2 and 3 (diabetic sera) well 4 (cholecystic sera) and well 5 (acute pancreatitis sera)

In the case of the sera of acute pancreatitis, total serum amylase activity was very high and agarose gel electrophoresis had only one band of P-type isoamylase, and the S-type isoamylase band was completely absent. Bossuyt *et al.* also got the same type of band patterns and reasoned out that P-type isoamylase contributed by the pancreas is very high and making S-type isoamylase negligible in comparison [10].

Diabetes mellitus is a common endocrine disorder. Total serum amylase activity of uncontrolled diabetic subjects had a slight elevation, and agarose gel electrophoresis showed faded and thin P-type isoamylase whereas S-type isoamylase band was prominent. It has been studied that the exocrine function of the pancreas becomes defective in uncontrolled diabetic subjects, and the pancreas cannot secrete sufficient amount of P-type isoamylase, which may be the reason for decreased P-type band in electrophoresis. Now-a-days treatment modalities have targeted for controlling postprandial hyperglycemia as proved by Sneha and Chaudhari with plant extracts studied α -amylase inhibitory activity in diabetic subjects [11,12].

The electrophoretic band patterns of sera of cholecystitis patients showed prominent P-type band when compared to decreased and faded S-type isoamylase band. In these patients, there occurs the leakage of P-type isoamylase enzyme that enters the circulation via peritoneal cavity. This may be the cause for increased P-type isoamylase electrophoretic band.

We also compared the electrophoretic band patterns with the normal sera band. Electrophoretic pattern was characteristic in acute

pancreatic patients. The serum amylase activity of uncontrolled diabetic and cholecystitis patients was moderately increased, but the isoamylase band patterns were different. Therefore, in conditions of a moderate rise of serum amylase enzyme activity it is useful to study isoamylase band patterns by electrophoresis that can lead to the next level of investigations. Further quantification of isoamylase enzyme bands by densitometer is more useful.

CONCLUSION

Agarose gel electrophoresis is a simple technique run with minimal efforts. Although we could not measure the bandwidth using a densitometer, we compared the band patterns between hyperamylasemic conditions. The study of different isoamylase band patterns helps in differentiation of the diseases that gives information of the defective organ and abnormal source of origin of serum amylase.

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