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Proteomics: A hallmark tool for identification of Biomarker (LIPASE) in type I & II diabetes mellitus patients.

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ABSTRACT

*Proteomics is an indispensable tool in present day research. It has become important specifically in identification of various proteins and biomarkers for diagnosis of various diseases. In the present proteomic study, a similar approach has been applied for identification of proteomic biomarker by 2-D Gel electrophoresis in diabetic patients. 100 blood samples withdrawn from diabetic patients attending diabetic clinic were analyzed for concentration of lipase and glucose and the serum samples were run on SDS-PAGE gel .**Result:** Lipase, glucose levels of diabetic patients were above normal range in insulin dependent diabetes mellitus patients (IDDM) and non-insulin dependent diabetes mellitus patients (NIDDM) and the results were statistically significant ($p < 0.0001$). Band pattern of SDS-PAGE showed that the band of lipase, superoxide dismutase (SOD), M-isoform-phosphofructokinase (M-PFK) of IDDM were intense (darker) than NIDDM and normal individuals. **Conclusion:** Lipase along with other metabolites i.e. SOD and M-PFK has been identified as biomarker in diabetes mellitus. Identified biomarkers can be of immense help for diagnosis of endocrinal dysfunction.*

Key words: Proteomics, lipase, SDS-PAGE gel, Insulin Dependent Diabetes Mellitus.

INTRODUCTION

Proteomics is the study of proteome i.e. protein expression measurement. It has made a significant place in the field of research and has become remarkable tool specifically in the diagnosis of various diseases. It has been defined as the study of protein properties (expression level, post-translational modification, interaction, etc.) on a large scale to obtain global, integrated view of disease processes, cellular processes, and networks at the protein level. The study of all proteins by a cell type or an organism is called “proteomics” [1]. Kashanchi emphasized importance of proteomics and its application of novel and mature protein profiling technologies including mass spectrometry, yeast two hybrids, isotope – coded affinity tags, 2-D differential gel electrophoresis, 2-D capillary electrophoresis and protein microassays in discovering disease biomarkers for diagnostic applications and identifying and validating new targets and disease pathways for discovery and development.[2].

Proteomics now refers to any procedure that characterizes large sets of proteins The most significant breakthrough in proteomics has been the use of mass spectrometric identification of gel-separated proteome, two dimensional (2D) gel electrophoresis for protein separation and identification, [3, 4].Many researchers in their extensive work have worked on latest technique involving 2-D electrophoresis for separation and mass spectrometry for positive identification [5]. Figeys also demonstrated the importance of proteomics and its technical development and its application in identification of various biomarkers [6].

Two- dimensional polyacrylamide gel electrophoresis (2-D PAGE) is directly co-related with evolving new science of proteomics. It is commonly used to separate complex mixture of proteins based on their iso-electric point (PI) and molecular weight (MW). The emerging field of biomarker has applications in diagnosis, staging, prognosis and

monitoring of disease progression [7]. Similar studies were also observed by various scientists which made them able to identify new disease markers [8, 9, 10, 11, 12, and 13].

In 2009, Schitt Mayer highlighted the importance of proteomics in lipid research.

Lipases are key players in hydrolysis of lipids and by proteomics these can be discovered further [14]. Similarly, in 2010 scientist of Graz University worked on the analysis of the lipolytic proteome of cultured human fat cells [15].

Here, in the present study, over viewing the importance of proteomics, we have carried some work with respect to diagnosis of diabetes mellitus . For this we had applied, a similar type of approach towards 2-D gel electrophoresis and from the serum protein soup enzyme markers were identified. Lipase enzyme along with, another two metabolites i.e. SOD and M-PFK-1 has also been identified which are predominantly present in IDDM patients and that can be considered as a biomarker for endocrinal dysfunction.

MATERIALS AND METHODS

2.1 Subjects:

The present study comprised of 90µ patients from diabetic clinic. The consent from the patient was taken who were enrolled for study and approval from ethics committee of the institute. Fasting blood samples were withdrawn from hyperglycemic patients and normal individuals. The samples were analyzed for lipase, glucose and SDS-PAGE Gel. A detailed clinical history was taken which was co-related with the IDDM and NIDDM patients and the insulin dose.

2.2 Chemical and Reagents:

Lipase enzyme, EC3.1.13, source *Aspergillus niger*, mol. wt 35KD was procured from HIMEDIA. The substrate P-nitro phenyl palmitate (PNP) (MW=377.52) was procured from Fluka. For gel-electrophoresis of lipase enzyme, the gel electrophoretic apparatus from Bangalore, Gene was used. Glucose levels were estimated by GOD-POD method, end point [16]. All other chemicals and solvents were procured from SD. fine of analytical grade.

2.3 Experimental Part

Measurement and Characterization of Lipase enzyme:

The measurements of lipase assay was done spectrophotometrically by quantifying the enzymatically produced P-nitro phenol and read at 410 nm. For this, Shimadzu double beam UV-VIS spectrophotometer UV-1601 (Shimadzu Corp., Kyoto, Japan) was used.Enzyme assay was based on the determination of appearance of P- nitro phenol at 30ºC and pH 7.5 [17]. The substrate used was P-nitro phenyl palmitate (MW=377.5).

SDS-PAGE (sodium dodecyl sulphate - Polycrylamide gel electrophoresis):

SDS-PAGE of 10X and 20X diluted normal individuals, non-insulin dependent patients and insulin dependent patients was run by adjusting it at an isoelectric point pH = 4.65 of lipase [18].

RESULTS AND DISCUSSION

Proteomics is the analysis of direct measurement of proteins in a given sample. 2-D – PAGE is directly co-related with evolving new science of proteomics. A similar clinical approach has been applied to identify the marker for the diabetic patients. Lipase has been identified and proved as a very significant biomarker for these patients. Lipase (triacylglycerol acyclhydrolase; EC 3:1:1.3) catalyzes the hydrolysis of ester linkages i.e. the long chain fatty acid (LCFA) of triglyceride (triacylglycerol) and glycerol [19].

Table 1: Comparison of Glucose and Lipase concentration in Normal Individual, patients suffering from IDDM and NIDDM

Groups	Normal Individual N=30	IDDM Patients N=30	NIDDM Patients N=30
Glucose conc(mg%) Mean±SD	93±2	435±3*	234±2▪
Lipase(µU/ml) Mean±SD	143±3	265±3	195±2.5

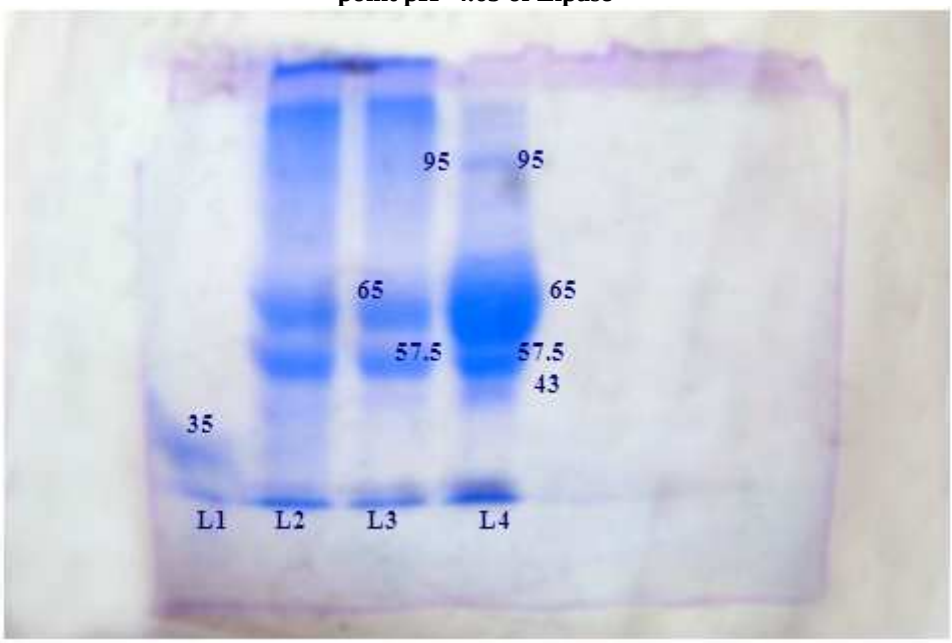
**p<0.0001,* *▪p< 0.0001*

Insulin stimulates lipogenesis and diminishes lipolysis. Lipase activity in IDDM and NIDDM is more as compared to normal healthy subjects. Lipases are involved in insulin secretion and the pharmacological inhibition of lipase

activity in β-cells impairs insulin secretion [20].The lipase activity was estimated in serum of normal healthy,patients sample from NIDDM/ IDDM) by PNP method [21].The method has been standardized with the synthetic lipase spectrophotometrically at 410 nm .

Table 1 shows the result and statistical analysis of glucose and lipase of normal individual, insulin dependent diabetic patients and non-insulin dependent diabetic patients. Lipase and glucose levels in IDDM and NIDDM patient were higher in comparison to normal individual with significant *p and ▪p value respectively which is true to the statement "Insulin inhibits lipolysis" [22, 23]. The 2D-gel has become an important tool for diagnosis, particularly by profiling the patient's serum. In this study, 2-D SDS/PAGE technique has been exploited for lipase activity. The serum samples of above mentioned groups were run on 2-D SDS page [18] at pH. 4.65 which is the IpH of lipase (for reconfirming the lipase activity).

Figure 1: SDS/PAGE OF (10X) Normal individual, NIDDM and IDDM patient by adjusting at isoelectric point pH=4.65 of Lipase



From Left to right
Lane 1 synthetic lipase
Lane 2 10x diluted Normal individual
Lane 3 10 x diluted NIDDM patient
Lane 4 10xdiluted IDDM patient

Table 2: SDS-PAGE Description of 10X

Lane 1	Lane 2	Lane 3	Lane 4
Synthetic Lipase (kDA)	Normal Individual (kDA)	NIDDM (kDA)	IDDM (kDA)
	95	95	95
	65	65	65(intense)
	57.5	57.5	57.5(intense)
	43	43	43(intense)
35			

The (Fig. 1/Table2) shows the different band positions and description of these band positions. The band positions at 65 kDa ,57.5 kDa and 43 kDa were intense (darker) in IDDM patients than NIDDM patients and normal individual. 65 kDa was the protein control i.e. BSA (bovine serum albumin). 57.5 kDa was identified as serum lipase and 43 kDa as ovalbumin. The band at position 43 kDa identified as ovalbumin was observed in IDDM patients only [29]. The ovalbumin protein is found in normal individual, NIDDM and IDDM patient. But the band at position 43kDa is more intense in IDDM patients than NIDDM and normal individual. The band positions reveal the identification of particular proteins in (Table 3).

Table 3: Expected Band pattern of particular proteins

Sl. no.	Mol. Wt. (kDa)	Analyte Identified	Reference
1	82.5	M-PFK-I (Phosphofructokinase Enzyme)	24
2	70	SOD(SuperOxide Dimutase)	25,26
3	66.0	Albumin	27
4	57.5 kDa	Human lipase	27,28
5	43 kDa	ovalbumin	27

In the second gel, the concentration of the sample was diluted to 20X (20times) so that band pattern of some proteins which were found to be overlapped in 10X, may not be missed. SDS PAGE of (20X) diluted Synthetic Lipase ,normal individual, IDDM and NIDDM patients was run and molecular weight of different observed bands were determined by comparing with a known standard marker PMW –H (29 -205 kDa) . Graph was plotted between electrophoretic mobility on X- axis in mm and log10 mol.wt on Y-axis. A standard curve was thus plotted and the molecular weights of unknown analytes were determined (Figure 2). The relationship of molecular weight and electrophoretic mobility relationship has been shown in (Table 4).

Table 4: Molecular weight and Electrophoretic Mobility Relationship

Sl. No.	Electrophoretic Mobility (mm)	Log_{10} Mol. Wt.
1	0.27	5.311
2	0.48	4.988
3	0.58	4.8195
4	0.80	4.633
5	0.94	4.462

Figure 2: Standard curve, for determining molecular weight and size of serum proteins

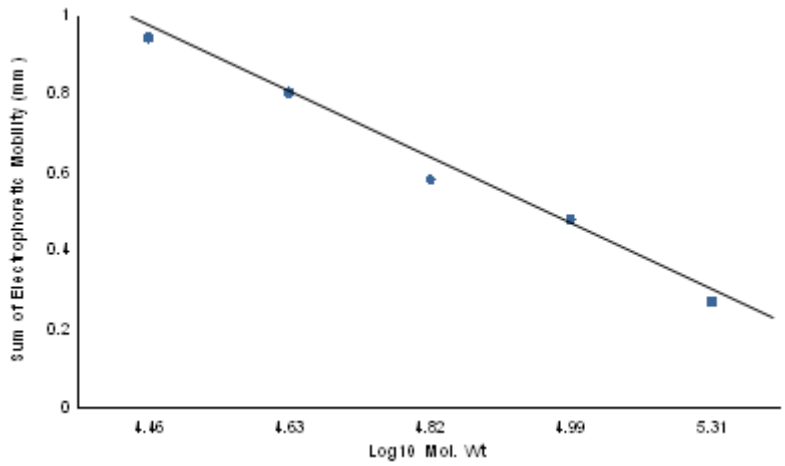
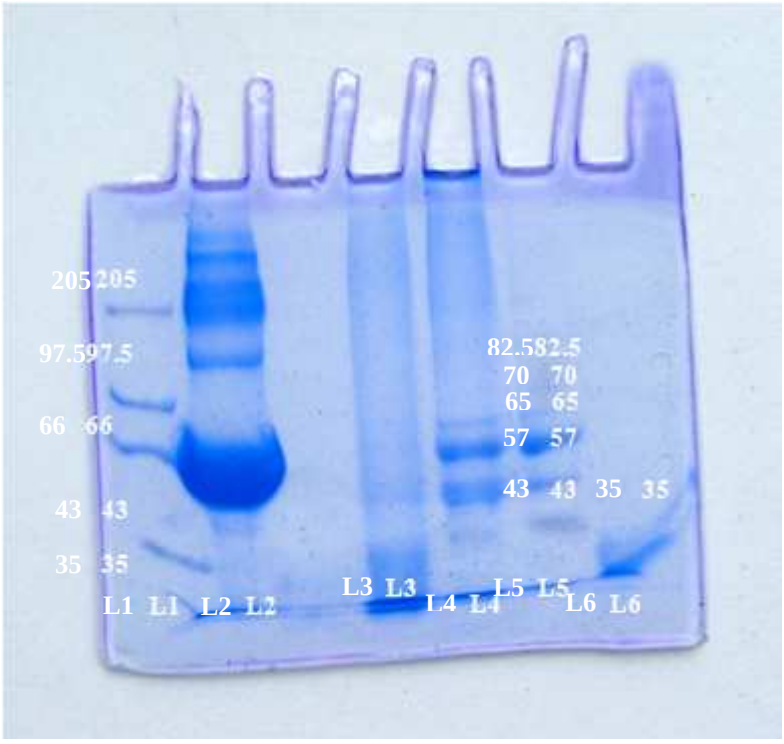


Table 5: SDS-PAGE Description of 20X

Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
PMW-H	BSA	Normal Individual	NIDDM	IDDM	Synthetic Lipase
205				82.5	
97.4		70(faint)	70	70	
66	66	65(faint)	65	65	
			57.5	57.5(intense)	
43			43	43(intense)	
29					35

Figure 3: SDS-PAGE of (20X) Normal individual, NIDDM and IDDM patient by adjusting at isoelectric point pH=4.65 of Lipase



Left to right
Lane 1 PML-H (29-205 kDa) – The marker
Lane 2 BSA Marker
Lane 3 20 x diluted normal individual
Lane 4 20 x diluted NIDDM Patient
Lane 5 20 x diluted IDDM Patient
Lane 6 Synthetic lipase

The Figure 3/Table 5 here shows the comparative band pattern and positions of the normal individuals, NIDDM and IDDM patients along with the known marker i.e. PML-H (29-205 KDA).In this ,20X SDS/PAGE gel, bands of molecular weight 82.5 kDa,70 kDa,65 kDa,57.5 kDa,43 kDa were obsereved. . The intensity of lipase(57.5 kDa) and ovalbumin(43 kDa) band were more intense in IDDM patients as compared to NIDDM patients and normal individuals The other distinct band pattern were also observed at position 82.5 kDa and 70kDa which were identified as (M-PFK) M- isoform of phosphofructokinase and (SOD) superoxide dimutase respectively . M-PFK enzyme participated in the control of the glycolytic flux and is raised in IDDM patients [24]. The SOD activity has been found to be elevated in the diabetic group [25, 26]. These recognized biomarkers seem to be significant for assessing the endocrinal dysfunction by proteomics means and had good co- relation clinically. These could be recognized as good biomarker for further study. The availability of individual genetic mapping will permit a reliable clinical evaluation and interpretation of tests of insulin secretion in the future [29].

CONCLUSION

The present study revealed the importance of proteomics in assessing the biomarker in the hyperglycemic patients specifically in IDDM patients. The lipase enzyme isolated from the human serum by SDS/PAGE had good significance in IDDM and NIDDM patients. Lipase activity was found to be increased in IDDM patients than NIDDM and normal individuals. Along with it, the M-isoform of phosphofructokinase and the antioxidant superoxide dimutase were also found to be higher in IDDM patients than NIDDM and normal individuals. This study of 2-D PAGE made a precise co-relation of the disease with the direct measurement of protein in a given sample at a given time. This 2-D PAGE has become important tool for assessing disorders with the association of evolving new science of “Proteomics”. Human plasma proteome inherits variable proteins soup. For further study, many biomarkers are yet to be identified in unknown protein soup.

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