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JOURNAL OF CLINICAL AND DIAGNOSTIC RESEARCH

JCDR doi: 189-126-280-287(published online first 18th July 2008)
Lipase And Ca\(^{2+}\) Ions As Biomarkers In Blood Serum Of NIDDM

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ABSTRACT
Non Insulin Dependent Diabetes Mellitus (NIDDM) is a metabolic disease characterized by insulin resistance, hyperglycemia, often associated with hypertension, lipid disturbances and obesity. Blood serum of normal healthy subject and NIDDM patients are considered for study. Lipase and Ca\(^{2+}\) ions concentrations have been compared in NIDDM and normal healthy subjects along with glucose concentration. Lipase activity is increased (287-325 U/L) in blood serum of NIDDM than normal healthy range (30-250 U/L). Ca\(^{2+}\) ions concentration has been decreased (1.5-3.4 mg/dL) below the normal range (8.9-10.4 mg/dL). Hence lipase can be used as a biomarker for disease diagnosis and low Ca\(^{2+}\) ions concentrations observed in NIDDM may be alarming leading to multiple diseases.

Key Word: Ca\(^{2+}\) ions, glucose, insulin, lipase and Non-Insulin Dependent Diabetes Mellitus.

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Introduction
Non Insulin Dependent Diabetes Mellitus (NIDDM) has become a major health problem worldwide. At present, there are about 125 million people affected by this disease, globally. Over 90% of cases of diabetes worldwide are in the form known as NIDDM. Insulin resistance is a prominent biological marker of NIDDM, particularly when it is associated with obesity, and it is defined as an impaired biological response to either exogenous or endogenous insulin. This response is not strictly confined to the relationship between insulin and glucose, but it extends to other actions of this hormone, including its effect on lipid and protein metabolism, vascular endothelium function and gene expression[7]. As a further proof of the importance of fat distribution as the determinant of insulin resistance by Sumo Wrestlers[8], lipids are found to play a complex role in glucose homeostasis under both normal and pathological conditions[2]. Lipases are involved in insulin secretion, and the pharmacological inhibition of lipase activity in β-cells impairs insulin secretion[9]. The Ca\(^{2+}\) ions are unique among all the mineral ion species due to their large intra extra gradient. Intracellular free Ca\(^{2+}\) ions are the determinants in regulating cardiac function and smooth muscle contraction, and act as a final common factor in the non cellular responsiveness to various stimuli[1]. Up to now, there have been no studies on lipase and Ca\(^{2+}\) ions as biomarkers in the blood serum of NIDDM. For this reason, we have now studied the lipase and Ca\(^{2+}\) ions concentrations in normal healthy subjects and NIDDM patients.

Materials and Methods
Selection of Samples
Samples were divided into two groups – Normal healthy subjects and NIDDM subjects. The blood serum of each group, aged around 35 – 45 years, was collected from the clinical laboratory, Patiala. Each group comprised of eight subjects who belonged to both male and female sexes. Samples were selected on the basis of age, as NIDDM manifests after age 40.

**Chemicals**

Lipase enzyme was procured from Hi media Laboratory Pvt. Ltd., Mumbai. All other chemicals and solvents of highest grade were procured from Loba Chemie. Pvt. Ltd., Sd fine, Mumbai.

**Equipment used**

Systronics UV- VIS Spectrophotometer119, Systronics Photofluorometer151 of Naroda, Ahmedabad, India.

**Glucose estimation**

Glucose levels were estimated in normal healthy subjects and NIDDM subjects by estimation of unreduced copper[11].

**Lipase activity**

Lipase activity was determined in normal healthy subjects and NIDDM subjects by measuring the formation of p-Nitrophenol from p-Nitrophenol palmitate[10]. SDS-PAGE of 20X diluted normal individual and NIDDM patients was run by adjusting it at an isoelectric point pH=4.65 of lipase[6]

**Ca\(^{2+}\) ions detection**

Concentration of Ca\(^{2+}\) ions in the blood serum of normal healthy subjects and NIDDM subjects was determined by developing the formation of a fluorescent chelate between Ca\(^{2+}\) ions and calcein in an alkaline solution. Fluorescence intensity measured at 540 nm with excitation at either 330 nm or 480 nm[12].

**Statistical Analysis**

Results are reported in triplicate as mean, which is a single value selected from a group of values to represent in some way – a value which is supposed to stand for the whole group of which it is part, as typical of all values in the group.

\[
\bar{X} = \frac{x_1 + x_2 + x_3 + \ldots + x_n}{n}
\]

**Results**

The glucose and lipase activity and concentration of Ca\(^{2+}\) ions are reported in [Table /Fig 1], [Table /Fig 2] , [Table /Fig 3] respectively.
Lipase activity was also confirmed by SDS–PAGE of 20X diluted normal individual and NIDDM blood serum by adjusting it at an isoelectric point pH=4.65 of lipase[6]. The lipase band found at position 57 kDa[4] was intense in NIDDM than in normal healthy subjects, as shown in [Table /Fig 1].

**Discussion**

Insulin stimulates lipogenesis and diminishes lipolysis. Cellular metabolism of fuel raises the ATP/ADP ratio, resulting in closure of K\text{ATP} channels and concomitant β-cell depolarization. This depolarization in turn, activates voltaged–gated Ca\textsuperscript{2+} channels, resulting in Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} dependent insulin exocytosis\(^5\). So on this basis, lipase and Ca\textsuperscript{2+} are selected as biomarkers. Lipase activity in NIDDM is higher than in normal healthy subjects, whereas concentration of Ca\textsuperscript{2+} ions is low in NIDDM than in normal healthy subjects. Lipases are involved in insulin secretion and the pharmacological inhibition of lipase activity in β-cells impairs insulin secretion. A low concentration of Ca\textsuperscript{2+} ions observed in NIDDM may be considered as alarming, leading to multiple diseases. Further studies are needed to establish this.

**Conclusion**

Concentration of Ca\textsuperscript{2+} ions had decreased (1.5-3.4 mg/dL) below the normal range (8.9-10.4 mg/dL). The lipase band found at position 57 kDa[4] was intense in NIDDM subjects than in normal healthy subjects. This study shows that lipase and Ca\textsuperscript{2+} ions are very important biomarkers as these can be used for disease diagnosis. Lipases are involved in insulin secretion and the pharmacological inhibition of lipase activity in β-cells impairs insulin secretion. A low concentration of Ca\textsuperscript{2+} ions observed in NIDDM may be considered as alarming, leading to multiple diseases. Further studies are needed to establish this.

**Acknowledgment**

The financial aid for the purchase of the instruments in the Department of Biotechnology, Punjabi University, Patiala, Punjab, by AICTE, is fully acknowledged.

**References**


[5] Khan AF, Goforth BP, Zhang M and Satin SL. Insulin activates ATP sensitive K\textsuperscript{+} channels in pancreatic β-cells though give rise to a no. of diseases which are common in the old age, such as hypertension, arteriosclerosis, neurodegenerative diseases, malignancy and degenerative joint disease[3].


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