ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Polymorphic studies in PDX1/IPF1 Gene a key factor for the regulation of Insulin Gene

Prasad.M.P[1*], Rekha Sethi[2] and Anand.M[1]

1. Department of Microbiology/Biotechnology, Sangenomics Research Lab, Domlur Layout, Bangalore 560071, India.

2. Director IQAC/ Professor, Department of Microbiology, Jain University, Bangalore, India

Manuscript Info

Manuscript History:

Received: 29 September 2014
Final Accepted: 29 October 2014
Published Online: November 2014

Key words:
PDX1, PCR, Insulin, Diabetes mellitus, polymorphism

****Corresponding Author***

Prasad.M.P

Abstract

Diabetes is a major public issue due to its high prevalence and long-term complications. The molecular pathogenesis of diabetes, however, remains largely unknown. Diabetes is a result of a failure of the B cells of the endocrine pancreas to produce the amounts of insulin required to dispose of glucose, resulting in elevated blood glucose levels. Insulin is of vital importance in maintaining glucose homeostasis in mammals. This, and its unique role as the only anabolic peptide hormone, necessitates strict regulation and fast-acting mechanisms guaranteeing efficient insulin biosynthesis and secretion. In the present investigation primer Forward 5'-GCCGCAGACAATGGACTC-3' and Reverse 5'-AGATGCCCTTGCTGCTGTCACC-3' were used to detect the polymorphism among different diabetic blood samples. The amplification of the PDX 1 gene resulted in the amplification of the particular gene sequence in few Samples and the control sample and the PCR product was found to be around 320 base pair long after comparing with the marker. A blood sample showed no bands on the agarose gel which shows polymorphism in the particular gene in the given sample.

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Introduction

Diabetes is a metabolic disorder where in human body does not produce or properly uses insulin, a hormone that is required to convert sugar, starches, and other food into energy. Insulin is a hormone that has extensive effects on metabolism and other body functions, such as vascular compliance. Insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle, and stopping use of fat as an energy source. Insulin is used medically to treat some forms of diabetes mellitus.

Pdx1 (Pancreatic and duodenal homeobox 1), also known as insulin promoter factor 1, is a transcription_factor necessary for pancreatic development and β-cell maturation. PDX-1 is a homeodomain-containing transcription factor which binds to the A element motif of the insulin genes and contributes to directing the genes' b cell–specific expression (German. M et al., 1995). PDX1 causes no change in the expression of beta cell-specific genes such as the insulin gene. Thus, the amount of PDX1 per session at least as long as it is above a certain level, and it is unlikely to primarily explain the decrease in insulin gene transcription caused chronic high glucose exposure.

PDX1 is a homeodomain-containing protein which is a key factor in the regulation of the insulin gene in pancreatic beta cells, and is critical for the development of the pancreas (Ohlsson. et al., 1993). IPF1 is a key factor both for the regulation of insulin gene expression and for the development of the pancreas. IPF1-containing stem cells are essential for beta-cell regeneration after injury in pancreatic islets (Fernandes et al., 1997). Decrease of the IPF1 content in beta cells exposed to supra physiological levels of glucose is considered to be a mechanism for glucose toxic effects on the beta cells leading to defective insulin secretion and worsening of hyperglycemia (Olson. et al., 1995). The reduced expression of PDX-1 responsive genes also results in the compromised glucose sensing found in mice and humans carrying only one functional pdx-1 allele. (Stoffers et al., 1997).

MATERIALS AND METHODOLOGY:

Sample collection:

Diabetic patient blood samples were used for the present study. These diabetic patient blood samples were collected from Vital Diagnostics and Multi-specialty Clinic, Domlur, Bangalore. The patient age was between 35-50yrs. Also one control sample from normal patient was used for comparison. The blood samples were stored at 4º C until use.

Isolation of Genomic DNA:

1 ml of blood was mixed with 4 ml of Solution A in an eppendorf tube. The blood sample was centrifuged at 10,000 rpm for 20 minutes. Supernatant was discarded and to the pellet 0.5 ml of reagent B and 0.125 ml reagent C was added and mixed well. Then 0.3 ml Phenol and 0.3 ml Chloroform and Isopropyl alcohol (24:1) mixture was added. The tube was centrifuged at 8000 rpm for 10 minutes. Three layers – bottom Phenol layer, middle Chloroform layer and upper aqueous layer were formed. The upper layer was taken and 0.3 ml of chloroform was added. The tube was centrifuged at 8000 rpm for 10 minutes. To the upper aqueous phase double the volume of ice cold ethanol was added and incubated at -20˚c for 20 min. The tube was centrifuged at 12,000 rpm for 10 minutes to remove the supernatant. The pellet was air dry and to it 80-100µl of TE buffer was added and was stored at 4ºC. The qualitative analysis was carried out with 1.0% agarose gel electrophoresis and quantitative estimation was done using Nanodrop 1000.

Polymerase Chain reaction:

The polymerase chain reaction was carried out for the isolated DNA with primers F-GCCGCAGACAATGGACTC and R-AGATGCCCTTGCTGCTGTCACC. The PCR conditions for the primer were standardised. The initial denaturation was carried out at 94º C for 4 min. Final denaturation at 94 ºC for 45 min, Annealing temperature was 55 ºC for 45 min and Extension was at 72 ºC for 45 min. This cycle was repeated for 30 cycles. The final elongation was carried out at 72 ºC for 10 mins. The PCR products were determined on 1.5% agaore gel.

RESULTS:

Qualitative analysis of DNA:

The DNA isolated from the blood samples were qualitatively analyzed on 0.8% agarose gel (Figure I).

Quantitative DNA estimation:

The quantity of DNA was estimated by Nanodrop spectrophotometer at 260/280nm, if the optical density value is between 1.6-1.8 the DNA is said to be in the pure form (Figure II).

Polymerase chain reaction:

PCR amplification of the PDX 1 gene resulted in the amplification of the particular gene sequence in Sample 1, 2, 3 and the control sample and the PCR product (amplicon) was found to be around 320 base pair long after comparing with the marker. Sample 4 showed no bands on the agarose gel shows no polymorphism indicating that the particular gene in absent in the sample (figure III).

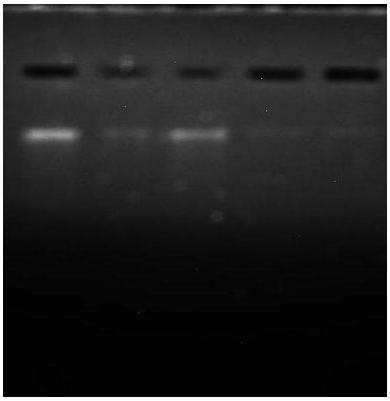

Figure I: Qualitative analysis of DNA from blood samples

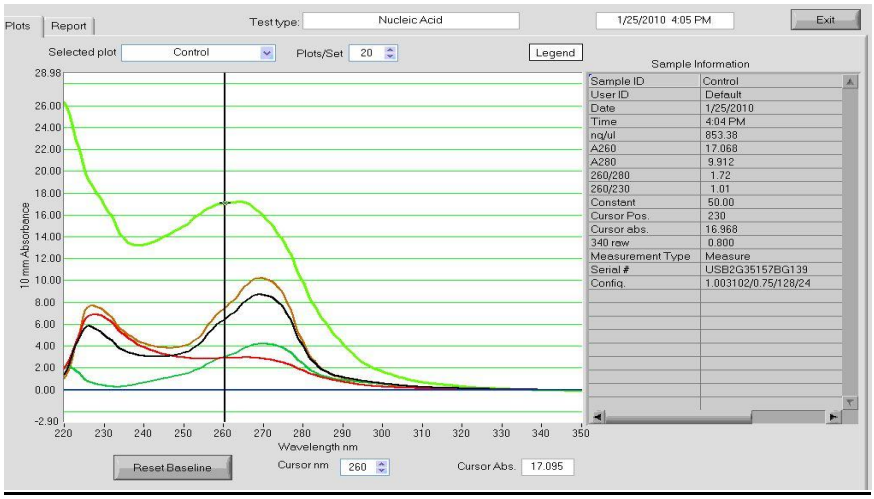

Figure II: Quantitative estimation of DNA from blood samples

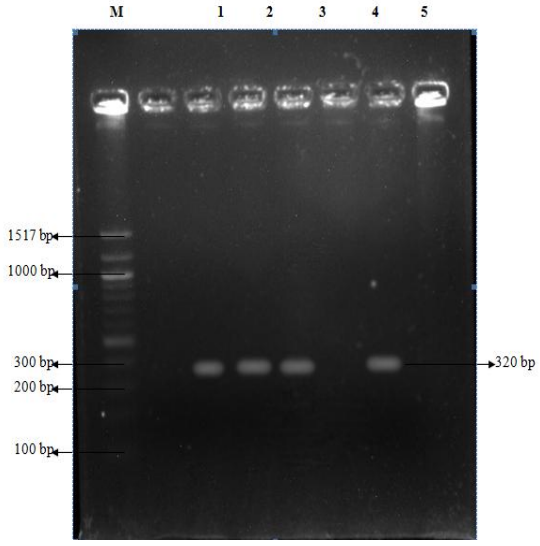

Figure III: PCR amplification of PDX1 gene from blood samples.

DISCUSSION:

The ability of the cell to provide insulin in sufficient amounts to meet the body's needs is compromised in type 2 diabetes mellitus patients with mutations in BETA2 (Malecki et al., 1999) and PDX-1 (Hani et al., 1999; Stoffers et al., 1997).

Pdx-1 have proved to have a direct impact on insulin transcription derived primarily from electrophoresis mobility shift analysis in vitro and studies of reporter gene expression in mammalian cells by many investigators (Liberzon, et al., 2004; Ohlsson et al., 1993; Le Lay et al., 2004; Ohneda et al., 2000; Peshavaria et al., 1994).

Yi Qiu et al., 2002, investigated the unique combination of PDX-1, BETA2, E47, and p300 and showed that they promote synergistic activation from a transfected insulin enhancer-driven reporter construct in non- cells, a process inhibited by E1A. In addition, E1A inhibited the level of PDX-1 and BETA2 complex formation in cells. PDX-1 has also been shown to bind directly to BETA2 and E47 by in vitro GST pull down analysis. Activator-activator interactions between PDX-1, BETA2, and E47 have been shown to promote cooperative DNA binding on insulin enhancer DNA in vitro 3.5-fold between E47 and PDX-1 (Ohneda et al., 2000).

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