DETECTION OF AUTOANTIBODIES GAD 65 USING REAGENT KIT ISOLATED FROM BOVINE BRAIN TISSUE FOR PRE DIABETES MELLITUS PATIENTS

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PRODUCTION OF ANTI - GLUTAMIC ACID DECARBOXYLASE AND ANTI - GAD ANTIBODIES FOR PREDICTION TYPE 1 DIABETES MELLITUS PATIENTS

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INTRODUCTION

One of the most sensitive and specific autoimmune marker that can be used to detect pancreatic beta cell destruction in Type I Diabetes is the autoantibodies to GAD 65 (anti-GAD65 Abs). Most of the pre-Type 1 diabetic patients showed reactivity to antiGAD65 Abs. For the early detection of anti-GAD65 Abs in the serum of Type 1 Diabetes patients, human recombinant GAD65 has been successfully used. However, this reagent is very expensive. Therefore, it is necessary to find the alternative source of cheaper Anti-GAD65 reagent for detection of anti-GAD65.

The aim of this research is to develop alternative for diagnosis of pre Type 1 Diabetic using brain tissue as source of GAD.

METHOD

GAD Enzyme isolated from Bovine Brain was characterized by measuring activity at optimum condition with and without his Activators and his Inhibitors. Molecular relative of GAD was identified by SDS-PAGE Method.

The characters of Anti GAD and Anti-GAD Antibodies with Induced by GAD and Anti-GAD respectively were determine Immunologically using Western and Dot Blotting.
BACKGROUND & OBJECTIVE

One of the most sensitive and specific autoimmune marker that can be used to detect pancreatic beta cell destruction in Diabetes Type I Diabetes is the *autoantibodies to GAD 65* (anti-GAD65 Abs). Most of the pre-Type 1 diabetic patients showed reactivity to antiGAD65 Abs. For the early detection of anti-GAD65 Abs in the serum of Type 1 Diabetes patients, human recombinant GAD65 has been successfully used. However this reagent is very expensive. Therefore it is necessary to find the alternative source of cheaper Anti-GAD65 reagent for detection of anti-GAD65.

The aim of this research is to develop alternative for diagnosis of pre Type 1 Diabetic using brain tissue as source of GAD.
β-cell function

100%

GAD65 antibodies
ICA antibodies
IAA antibodies
IA2 and IA-2β

Abnormal β-cell function test
Tolerance reestablished

T-cell tests (+)

T-cell tests (–)

5-10%

Time (Months to years)

Clinical onset

(Mehta et al, 1996)
β-cell function

- GAD65 antibodies
- ICA antibodies
- IAA antibodies
- IA2 and IA-2β

Abnormal β-cell function test

Tolerance reestablished

T-cell tests (+)

Clinical onset

T-cell tests (–)
THE NATURAL HISTORY OF TYPE 1 DIABETES MELLITUS
(WHO Study Group, 1994)

Prevention

Autoimmune markers

Complications

Onset of diabetes

Primary

Secondary

Tertiary

Fetal development

Prediabetes

Diabetes

ICA (+)

IAA (+)

Anti GAD65Abs

Anti IA-2

Anti IA-2β

Absent

Maybe present

Increasingly frequent

Death

Insulin dependency

Hyperglycaemia

Insulin dependency

Increasingly frequent

Autoimmune markers

Complications

The natural history of type 1 diabetes mellitus is illustrated in the diagram. The stages are categorized into prevention, primary, secondary, and tertiary, each with specific markers and complications.

- **Prevention**
  - Autoimmune markers
  - Complications

- **Primary**
  - Fetal development
  - Prediabetes
  - Onset of diabetes

- **Secondary**
  - Diabetes
  - Hyperglycaemia

- **Tertiary**
  - Insulin dependency
  - Increasingly frequent complications

This diagram outlines the progression from fetal development to the onset of diabetes and subsequent complications, highlighting the role of autoimmune markers and the increasing frequency of complications over time.
THE NATURAL HISTORY OF TYPE 1 DIABETES MELLITUS
(WHO Study Group, 1994)

Prevention

Primary
- Onset of diabetes
- Fetal development
- Prediabetes
- ICA (+)
- IAA (+)
- Anti GAD65Abs
- Anti IA-2
- Anti IA-2β
- Absent

Secondary
- Diabetes
- Hyperglycaemia
- Insulin dependency
- Maybe present

Tertiary
- Death
- Increasingly frequent

Autoimmune markers

Complications

Insulin dependency

Death
MATERIAL & METHOD

Isolation, and Characterization of GAD65 from bovine brain

Production, Purification and Characterization Anti-GAD65 and anti-GAD65-antibody as Primary and Secondary antibody

Labeling anti-GAD65-antibody: analysis immunologically using Western-Blotting and Dot-Blotting Methods

PRODUCTION OF GAD 65, ANTI-GAD 65 AND ANTI-GAD 65 ANTIBODY AS REAGENT DETECTION FOR PRE DIABETES MELLITUS PATIENTS
### Biochemical Characters of GAD enzyme

<table>
<thead>
<tr>
<th>ITEM</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum Conditions (pH, Temp, Time Incubation)</td>
<td>7, 37°C, 15 minutes</td>
</tr>
<tr>
<td>Km, Vm</td>
<td>3.4626 unit, 7.663 x 10^{-3} M</td>
</tr>
<tr>
<td>Specific Activity of Purified GAD: Ammonium Sulphate Sephadex G-75</td>
<td>4.6623 units/mg, 20.400 units/mg</td>
</tr>
<tr>
<td>Relative Molecular weight</td>
<td>65 kDa</td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Zn^{2+}, Fe^{2+}, Cu^{2+}</td>
</tr>
<tr>
<td>Activators</td>
<td>Pyridoxal Phosphate (PLP)</td>
</tr>
</tbody>
</table>

**FIGURE 1: Electrophoregram of GAD, Anti-GAD65 and Anti-GAD65 Antibody**
FIGURE 2: Electrophoreogram of Anti-GAD65 Antibody prepared by Electroelution Method

FIGURE 3: Electrophoreogram of GAD, Anti-GAD65 and Anti-GAD65 Antibody
Figure 4: Result of Dot Blotting Method using GAD65 in variation concentration with constant concentration of Anti-GAD65 Antibodies AP Conjugate

Figure 5a: Result of Dot Blotting Method using GAD65 in variation concentration with constant concentration of Serum Human & Rat (DM & Kontrol) and Anti-GAD65 Antibodies Peroxidase Conjugate

Figure 5b: Result of Dot Blotting Method using Serum Human (DM & Kontrol) and Serum Rat DM with In variation concentration with constant concentration of GAD65 and Anti-GAD65 Antibodies Peroxidase Conjugate
THANK YOU...
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