THE EFFECT OF PANCREATIC INSUFFICIENCY ON HYPOGLYCEMIA AND GLUCAGON RESPONSE IN CHILDREN WITH CYSTIC FIBROSIS

Short Running Title: Hypoglycemia and Glucagon Response in CF

Belma Haliloglu, MD,PhD [1,2]
Tuba Seven Menevse, MD [1]
Seda Gulec Yilmaz, PhD [2]
Tuba Akdeniz, PhD [2]
Busra Gurpinar Tosun, MD [1]
Serap Demircioglu, MD [1]
Tulay Guran, MD [1]
Yasemin Gokdemir, MD [3]
Ela Erdem, MD [3]
Bulent Karadag, MD [3]
Turgay Isbir, MD, PhD [2]
Abdullah Bereket, MD [1]

[1] Division of Pediatric Endocrinology and Diabetes, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey
[2] Department of Molecular Medicine, School of Health Science, Yeditepe University, Istanbul, Turkey
[3] Division of Pediatric Pulmonogy, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey

Corresponding author: Belma Haliloglu, belma.haliloglu@marmara.edu.tr
belmahaliloglu26@hotmail.com

Date of document: 30/12/2022

STUDY PROTOCOL

1.Introduction

The exact underlying mechanism of hypoglycemia in CF is still unknown. Some recent studies support the delayed and prolonged insulin secretion and impaired counterregulatory hormone response as the reason of reactive hypoglycemia, whereas the others argued an additive effect of an intrinsic factor. However, the weakness of these limited studies is that nearly all of them included CF patients who had pancreatic insufficiency (PI) and could not reveal the mechanism of hypoglycemia seen in those without PI. In addition, there were no healthy controls for comparison of glucagon secretion in CF patients with hypoglycemia. Moreover, the studies that evaluate the role of glucagon in hypoglycemic CF patients were performed in hypoglycemic adult patients with abnormal glucose tolerance (AGT) and the delayed and prolonged insulin release is expected to be more likely as the reason of hypoglycemia in this setting. Previously, the investigators had demonstrated isolated hypoglycemia in some of the pediatric CF patients during OGTT. In this study, they further evaluated possible mechanisms of hypoglycemia. The investigators hypothesized that the mechanism of isolated hypoglycemia might be different from hypoglycemia seen in patients with AGT. Furthermore, they investigated the role of pancreatic insufficiency in hypoglycemia of CF patients by analyzing glucose, insulin and glucagon response to a glucose load in CF patients with an without PI.

2. Methods

2.1. Participants

Children and adolescents aged 10-18 years with CF who were regularly followed-up at the Pediatric Pulmonology and Endocrinology Units, between January 2020 and December 2020 were invited to participate in the study before their annual OGTT. Pancreatic insufficiency (PI) was defined by need for enzyme replacement therapy based on clinical symptoms or fecal elastase level (<200 μg/g). Individuals using corticosteroid therapy, those who had acute exacerbation in the last 3 months or previously diagnosed with diabetes were not eligible. Height, weight, BMI, FEV1, genetics and the presence of PI of the participants were recorded.

The control group included age-matched healthy, non-diabetic siblings of patients with type 1 diabetes who were followed-up at our clinic. All the controls were negative for β-cell autoantibodies (anti-glutamic acid decarboxylase, islet cell antibody, and insulin antibody). The study protocol was approved by the Marmara University Ethics Committee (no:09.2019.933), and written informed consent was obtained from the parents and the participants.

2.2. Procedures

A 3-h OGTT was performed in the morning following overnight fasting of ≥8 h. All participants (CF patients and controls) received oral glucose solution (1.75 g/kg; max: 75 g). Blood samples were collected at 0, 30, 60, 90, 120, 150 and 180 min for glucose and insulin, 0, 60, 120,150 and 180.min for glucagon measurement. HbA1c and CRP levels at baseline and cortisol levels at 0-180.min were also measured to assess the status of inflammation and body stress.

OGTT results were categorized into different subgroups of glucose tolerance according to ISPAD guidelines (12). Subjects who had INDET (Indeterminate Glucose Tolerance) or IGT (Impaired Glucose Tolerance) without hypoglycemia on OGTT were classified

as Abnormal Glucose Tolerance (AGT). According to the IHSG position statement, hypoglycemia was defined in the presence of any venous glucose level is lower than 70 mg/dL (13). The terminology of "isolated hypoglycemia" (IsoHypo) was used for the participants who had hypoglycemia without AGT or CFRD and "Hypo+AGT" was used for the participants who had hypoglycemia with AGT. After the first analyses, the participants with IsoHypo and NGT were also sub-classified according to the presence of PI to evaluate the effect of PI on hypoglycemia (Figure-1).

2.3. Plasma Glucose, Insulin, Cortisol and Glucagon Analysis

Blood samples of the participants were collected in EDTA tubes and centrifuged immediately after collection. Glucose, insulin, cortisol and CRP were measured in the same day in the laboratory at Marmara University Hospital. Glucose was measured by glucose hexokinase method (Cobas c701/702, Roche). Insulin and cortisol were measured by ECLIA (Cobas e801 Roche and e601 Roche, respectively. For glucagon samples, plasma was separated after centrifugation, and stored at 4°C until the OGTT was ended (180 min). Following the completion of OGTT, all glucagon samples were immediately stored at -80°C prior to shipment to the laboratory at Yeditepe University where glucagon assay was performed.

Plasma glucagon was measured by using a direct sandwich ELISA technique (Mercodia Glucagon ELISA, cat. no. 10-1271-01, lot no. 29870, Uppsala, Sweden) with standard manufacturer's protocol. Optical density (OD) values of the samples were read in ELISA plate reader at 450 nm wavelengths (EPOCH-Spectrophotometer, BioTek, USA). The concentrations of the samples were calculated according to the standard curve drawn based on the OD of the manufacturer's standards with known concentrations. Each sample was measured in two different wells for double-check.

Commercial controls pools with low, intermediate and high glucagon concentrations routinely assayed as samples.

THE EFFECT OF PANCREATIC INSUFFICIENCY ON HYPOGLYCEMIA AND GLUCAGON RESPONSE IN CHILDREN WITH CYSTIC FIBROSIS

Short Running Title: Hypoglycemia and Glucagon Response in CF

Belma Haliloglu, MD,PhD [1,2]
Tuba Seven Menevse, MD [1]
Seda Gulec Yilmaz, PhD [2]
Tuba Akdeniz, PhD [2]
Busra Gurpinar Tosun, MD [1]
Serap Demircioglu, MD [1]
Tulay Guran, MD [1]
Yasemin Gokdemir, MD [3]
Ela Erdem, MD [3]
Bulent Karadag, MD [3]
Turgay Isbir, MD, PhD [2]
Abdullah Bereket, MD [1]

[1] Division of Pediatric Endocrinology and Diabetes, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey
[2] Department of Molecular Medicine, School of Health Science, Yeditepe University, Istanbul, Turkey
[3] Division of Pediatric Pulmonogy, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey

Corresponding author: Belma Haliloglu, belma.haliloglu@marmara.edu.tr
belmahaliloglu26@hotmail.com

STATISTICAL ANALYSIS METHOD

All analyses were carried out using GraphPad Prism® V5.0 software (GraphPad Software Inc., San Diego, California, USA). Variables are summarized for each group using descriptive statistics as percentages and mean±SD. Pairwise comparisons were performed using the Student T test or the Mann-Whitney U test depending on the data's normality. Kolmogorov-Smirnov test was used to evaluate of the data normality. Data were expressed as mean and standard error or deviation, which was stated. Statistical significance was set at $p < 0.05$.

THE EFFECT OF PANCREATIC INSUFFICIENCY ON HYPOGLYCEMIA AND GLUCAGON RESPONSE IN CHILDREN WITH CYSTIC FIBROSIS

Short Running Title: Hypoglycemia and Glucagon Response in CF

Belma Haliloglu, MD,PhD [1,2]
Tuba Seven Menevse, MD [1]
Seda Gulec Yilmaz, PhD [2]
Tuba Akdeniz, PhD [2]
Busra Gurpinar Tosun, MD [1]
Serap Demircioglu, MD [1]
Tulay Guran, MD [1]
Yasemin Gokdemir, MD [3]
Ela Erdem, MD [3]
Bulent Karadag, MD [3]
Turgay Isbir, MD, PhD [2]
Abdullah Bereket, MD [1]

[1] Division of Pediatric Endocrinology and Diabetes, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey
[2] Department of Molecular Medicine, School of Health Science, Yeditepe University, Istanbul, Turkey
[3] Division of Pediatric Pulmonogy, Department of Child Health and Disease, Marmara University School of Medicine, Istanbul, Turkey

Corresponding author: Belma Haliloglu, belma.haliloglu@marmara.edu.tr
belmahaliloglu26@hotmail.com

INFORMED CONSENT FORM

Title of the Study: The Effect Of Pancreatic Insufficiency On Hypoglycemia And Glucagon Response In Children With Cystic Fibrosis

Principal Investigators
Tuba Seven Menevse, MD
Department of Pediatric Endocrinology and Diabetes
Basibuyuk Mah. Marmara University Hospital, 34854, Maltepe-İstanbul / Turkey
+90542 553 8337
tuba.seven@hotmail.com

Belma Haliloglu, MD,PhD
Department of Pediatric Endocrinology and Diabetes
Basibuyuk Mah. Marmara University Hospital, 34854, Maltepe-İstanbul / Turkey
+90505 267 0197
belma.haliloglu@marmara.edu.tr

PURPOSE OF STUDY

You are being asked to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. Please read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information.

Hypoglycemia can be seen in patients with Cystic Fibrosis (CF). In these patients, the production and secretion of insulin hormone is diminished and dysregulated. However, the role and effect of glucagon in hypoglycemic patients with cystic fibrosis is still unknown. In this study, we aimed to investigate the frequency and etiopathogenesis of hypoglycemia in CF.

STUDY PROCEDURES

Oral glucose Tolerance Test (OGTT) is performed annually in all patients with Cystic Fibrosis. You will be also performed the OGTT test today, which should be performed

routinely. During OGTT, extra blood samples at the 0-60-120-150-180.min for glucagon analysis and at 0.min for CRP will be obtained in addition to the normal blood sampling. No additional procedure will be made for this study.

RISKS

In this study, no alternative treatment will be implemented and no additional procedure will be made. Therefore, there is no risk to you and does not cause any discomfort. You may decline to answer any or all questions and you may terminate your involvement at any time if you choose.

BENEFITS

There will be no direct benefit to you for your participation in this study. However, we hope that the information obtained from this study may help to understand the reason of hypoglycemia in CF.

CONFIDENTIALITY

Your data and results in this study will be anonymous. Every effort will be made by the researcher to preserve your confidentiality including the following:

- ✓ Assigning code numbers for participants that will be used on all research notes and documents
- ✓ Keeping notes and any other identifying participant information in a locked file cabinet and in a private computer of the researcher.

Participant data will be kept confidential except in cases where the researcher is legally obligated to report specific incidents.

CONTACT INFORMATION

If you have questions at any time about this study, or you experience adverse effects as the result of participating in this study, you may contact the researcher whose contact information is provided on the first page. If you have questions regarding your rights as a research participant, or if problems arise which you do not feel you can discuss with

the Primary Investigator, please contact the Department of Pediatric Endocrinology and Diabetes in Marmara University Hospital, 34854, Maltepe-İstanbul / Turkey

VOLUNTARY PARTICIPATION

Your participation in this study is voluntary. It is up to you to decide whether or not to take part in this study. If you decide to take part in this study, you will be asked to sign a consent form. After you sign the consent form, you are still free to withdraw at any time and without giving a reason. Withdrawing from this study will not affect the relationship you have, if any, with the researcher. If you withdraw from the study before data collection is completed, your data will be returned to you or destroyed.

CONSENT

I have read and I understand the provided information and have had the opportunity to ask questions. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and without cost. I understand that I will be given a copy of this consent form. I voluntarily agree to take part in this study.

Participant's signature _____ Date _____

Investigator's signature _____ Date _____