jcrpe-2019-0084.R1

Case Report

DOI: 10.4274/jcrpe.galenos.2020.2019.0084

The effectiveness of Sirolimus treatment in two rare disorders with nonketotic hypoinsulinemic hypoglycemia: The role of mTOR pathway

Runinng Title: Sirolimus treatment in nonketotic hypoinsulinemic hypoglycemia

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What is already known on this topics?
Nonketotic-hypoinsulinemic hypoglycemia is a very rare problem of glucose consumption increase without hyperinsulinism. In these cases, no effective therapy was implemented in addition to frequent feeding to counter hypoglycemia.

What this stıudy adds?
Sirolimus treatment could be a lifesaving tool for those kind of disorders as it appears to be effectively controlling the peristent hypoglycemia in NkHH, by causing mTOR inhibition.

Abstract
"Nonketotic-hypoinsulinemic hypoglycemia (NkHH)" is a very rare problem of glucose consumption increase without hyperinsulinism. This disorder has mainly been reported in cases with *AKT2* mutation and rarely in cases with *PTEN* mutation. In cases with *PTEN* or *AKT2* mutation, no effective therapy has been implemented in addition to frequent feeding to counter hypoglycemia.
mTOR inhibitor Sirolimus has been used in hyperinsulinemic hypoglycemia that was unresponsive to other medical treatment. In insulin signaling pathway, both AKT2 and PTEN play a role before mTOR. However, the role of Sirolimus on hypoglycemia in *AKT2* and *PTEN* mutations is unknown.
Case 1: Six monts old female with AKT2 mutation (c.49G>A (p.E17K) has showed NkHH. Frequent feeding was unsuccesful for treating the hypoglycemia and proptosis has been getting worse. Sirolimus treatment has been started at 3 years of age. Resultantly, blood glucose (BG) levels have been increased to normal levels.
Case 2: In a male case with *PTEN* mutation (p.G132V (c.395G>T), Persistent NkHH has appeared at 16 years of age (fasting BG: 27 mg/dl, fasting insulin1.5 mmol/L, while ketone negative). Sirolimus treatment was started and hypoglycemia was succesfully controlled.
NkHH is a very rare and significant disorder which provided some challanges in both diagnosis and treatment. Additionally, *AKT2* and also *PTEN* mutations could lead to NkHH.
Sirolimus treatment, by mTOR inhibition, appeared to be effectively controlling the peristent hypoglycemia and could be a lifesaving tool for these kind of disorders.
Keywords: AKT2, PTEN, Sirolimus, Hypoglycemia, treatment

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Submitted: 17-Jun-2019
Accept: 18-Feb-2020
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Introduction
Recurrent/pesistent fasting hypoglycema is a lifethreatening condition in childhood and frequently related to either hyperinsulinism or inborn errors of metabolism impairing liver glucose production (1). Hyperinsulinism is the most common cause of persistent hypoketotic, hypofattyacidaemic hypoglycaemia in infancy and childhood. In this situation, the excessive insulin secretion suppresses the mobilisation of fatty acids from adipose tissue, preventing ketone body synthesis in the liver (2, 3). Another well known cause of nonketotic hypoglycemia is fatty acids oxidation defect (4).
Recently, a few cases with unexplained, recurrent and severe fasting hypoglycemia without hyperinsulinism or fatty acids oxidation defects have been reported (1,5,6,7). We prefered to use the term of "Nonketotic-hypoinsulinemic hypoglycemia (NkHH)" in these cases.
NkHH is a very rare problem of glucose consumption increase without hyperinsulinism. In 2011, the first case with genetic defects of AKT2 lead to NkHH has been published. AKT2 is a serine/threonine kinase that plays an important role in insulin signal transduction. (7). Normally, when insulin combines with its receptor at target tissue, it requires phosphatidylinositol-3,4,5-trisphosphate (PIP3) to accumulate at the membrane plasma to facilitate its transmission within the cell. Gain of function mutation of AKT2 causes PIP3 accumulation without the need for insulin. Biochemichal profile of AKT2 activating mutation is very similar to hyperinsulinism (7).

Similar to AKT2 activating mutation, a defect in other molecules that have a role in insulin signalling has been expected to be cause of NkHH. Rarely, NkHH can also be developed with the mutation of a tumor suppressor gene called *PTEN* (8).

Treatment of hypoglycemia in NkHH can be very difficult, because there is no benefit of medical therapy against insulin synthesis or secretion. In those cases, no effective therapy has been implemented in addition to frequent feeding to prevent hypoglycemia. Besides, there is no therapetic implementation in case patients can not be fed for reasons such as; vomiting, gastrointestinal problems, anorexia etc.
The mammalian target of rapamycin (mTOR) inhibitor Sirolimus has been used in hyperinsulinemic hypoglycemia which was unresponsive to other medical treatment (3,9). In the insulin signaling pathway, both AKT2 and PTEN play a role before mTOR. However, the role of Sirolimus on hypoglycemia in *AKT2* and *PTEN* mutations is unknown.
In this paper, clinical and biochemical characteristics of two rare cases with NkHH has been presented. Additionally, the effect of Sirolimus on hypoglycemia has been reported in these cases.

Cases

Case 1: Seven months old female patient has been brought to the clinic by her family because she had recurrent hypoglycemia for 1 month. She was born at term with a history of polyhydroamnios. On physical examination; bilateral proptosis, hypertrichosis, hypertelorism, flat nasal bridge, macroglossia and achantosis nigricans have been noticed. At the time of admission; her height was 68 cm (50th percentile) with a weight of 7700 g (25-50th percentile) and a head circumference of 44 cm (75th percentile). She had hypoinsulinemic (<0.2 mIU/mL, C-peptide <0.1 ng/mL) and nonketotic during hypoglycemia (Blood glucose level was 27 mg/dL). Other biochemical and hormonal analysis showed normal results. Due to hypoglycemia occurence during fasting, frequent feeding in addition to cornstrarch to foods has been implemented. Whenver a severe hypoglycemia occured, iv glucose infusion has been given.
Genetic analysis revealed the de novo *AKT2* mutation (c.49G>A (p.E17K) in the patient (5). During follow-up, frequent feeding was unsuccesful for treating all the hypoglycemic episodes. Clinically, achantosis nigricans and proptosis have been getting worse (Figure 1). After informed consent was given by her parents, Sirolimus treatment has been started at 3 years of age. With the treatment, blood glucose (BG) levels increased to normal levels (mean BG before treatment: 48-52 mg/dl/day, after treatment 77-108 mg/dl/day). Neurological evaluation revealed normal language, cognitive, social, and fine motor development with a slight delay in gross motor development.
Case 2: A male case with multiple systemic involvement has been diagnosed with tumor hamartomatous syndrome before his admittance to our endocrinology department. He had verrucous epidermal nevus and adrenal hemorrhage at birth, renal vein and inferior vena cava thrombosis with hypertension at 5 months. At 16 months of age, he developed pelvic and retroperitoneal lipomatosis, multipl polyps of the colon and focal segmental glomerulosclerosis. Total colectomy for polyps has been caried out due to the recurrent bleeding. He also had macrocephaly, delayed motor mental development and epilectic seizures (Figure 2). These symptoms have suggested PTEN hamartoma-tumor syndrome (PHTS), so mutation analysis was conducted. Results revealed a *PTEN* mutation (p.G132V (c.395G>T) (10). Due to the *PTEN* mutation, he carried a high risk of thyroid malignancy. In this case, prophilactic thyroidectomy and LT4 replacement have been conducted.
During the follow-up of the patient, at 16 years of age, severe recurrent hypoglycemia (Blood glucose level: fasting BG: 6 to 27 mg/dl) has been noticed. Fasting insulin was low (1.5 mmol/L), while the ketone was negative. Hypoglycemia was persistent even though there was no known causes of hypoglycemia, with no detection of congenital metabolic disorders. We decided that patient had NkHH and frequent feeding has been offered, but this was not effective to resolve hypoglycemic attacks. Further from that, feeding was not possible due to the occasional anorectic periods of patient.
We were aware that PTEN was one of the molecules that may have a role in the insulin signalling mechanism. Additionally, it has been reported that, a few patients with *PTEN* mutation also had hypoyglycemic events.
After informed consent was given, Sirolimus treatment has been started on the patient. Folllow-up examinations and evaluations have been done on 3 monthly intervals by measuring the complete blood count, serum BUN, creatinin, electrolytes, AST, ALT, Lipid profiles, and HbA1c. Prior to starting Sirolimus treatment, Case 1 could not be able to fast longer than 3 hours. This time has increased to 4 to 5 hours with the treatment. A similar effect has been observed in Case 2 as well. Fasting time for Case 2 has increased to 3 to 4 hours, up from no more than 2 hours.
To expand upon individual cases, Case 1 hypoglycemia has been controlled successfully, in addition to the decreased feeding frequency. BG levels have been increased to normal levels (mean BG before treatment: 48-52 mg/dl/day, after treatment 77-108 mg/dl/day) with Sirolimus. After treatment, neurological evaluation has revealed normal language, cognitive, social, and fine motor development with a slight delay in gross motor development.
Case 2 required an increased dose of Sirolimus during the first month of the treatment. With increased dosage, the frequency and severity of hypoglycemia has been reduced (mean BG before treatment: 46-64 mg/dl/day, after treatment 62-92 mg/dl/day. Lowest fasting glucose level of 32 mg/dl has also been recorded for Case 2.
For both patients, begining dose of Sirolimus was 0.5 mg/m2/day. Dose of Sirolimus was titrated according to the serum level for both patients (between 4 to 12 mg/dl), and it has been increased to 1 mg/m2/day and 4 mg/m2/day in Case 1 and Case 2 respectively. Transient leucocytosis has been observed in Case 1 without any additional evidence. Duration of Sirolimus treatment wase 42 months in Case 1 and 9 months in Case 2.

Discussion

It is well known that, glucose homeostasis is maintained by the action of insulin on muscle, adipose tissue and liver (5). Insulin stimulate energy storage and growth through effects on glucose, lipid and amino acid metabolism. At cellular level, insulin effects are mediated by a transmembrane tyrosine kinase receptor that phosphorylates Insulin Receptor Substrate (IRS) and other adaptor proteins. Further on that, insulin signaling leads to activation of AKT serine/threonine kinases (1). In recent times, NkHH cases which resulted from activation of insulin signaling pathway have started to be published. *AKT2* mutation has been shown to be leading to this specific condition (1,8,11).

AKT2 is critical to control glucose and lipid metabolism. It is recruited to the cell surface by Phosphoinositide 3-kinase (PI3K) and phosphorilated by the Pyruvate Dehydrogenase Kinase 1 (PDK1) and mTOR c2 kinases. It has a transducer effect of insulin signaling to GLUT4 (12). The causes of hypoglycemia in *AKT2* mutation is related to the activation of insulin signalling pathway. First case with a gain of function of *AKT2,* which causes hypoinsulinemic hypoglycemia, has been reported in 2011 (1). Since then, a few cases have been reported, including ours (5,6,7).

AKT2 is a signal transducer in both glucose metabolism and lipid homeostasis (12). In our case, extraocular adipose tissue expantion leading to proptosis was prominent. Some adipose tissues could be impacted more from AKT2 mutation than others. The cause of this difference is unknown. An explanation to this could be the different expression levels of AKT2 in metabolic tissues (12).

PTEN is one the most important tumour supresors. Deactivation of it causes the activation of mTOR c1, then leads to the augmented translocation of specific mRNAs which is crucial for cell growth and proliferation (13). The PTEN–PI3K–AKT–mTOR pathway has a central role in the regulation of glucose metabolism. This pathway have downstream effects on the insulin receptor (INSR) and IRS adaptor molecules. It is known that the PI3K–AKT pathway enhances insulin-mediated glucose uptake and membrane translocation of the gluose transporter GLUT4, and inhibits gluconeogenesis (13). PTEN deficiency results in enhanced activation of the AKT signaling pathway. The mutation of PTEN augments PI3K signaling to AKT, then AKT signaling acts on mTOR pathway (14).

Usually, hypoglycemia in a patient with *PTEN* gene mutation does not come to attention. Schmid GL et al reported a case with PHTS, which was caused by germ line mutations in the *PTEN* gene. Case was treated with Sirolimus for uncontrolled tumor cell proliferation. In that case, a fasting glucose level of 1.9 mmol/l (35 mg/dl; reference: 3.6–5.6 mmol/l) was detected at the age of 42 months, although there was no extra information about the course of hypoglycemia (8). In our PTHS patient (Case 2), hypoglycemia has been detected at 16 years old. Blood sugar levels of this patient were very low at tiems and frequent feeding was ineffective to resolve hypoglycemia. In particular, the management of hypoglycemia in anorectic period of patient has been difficult. This situation led us to look for another treatment modalities.

PTEN loss induces adipogenic-like transformation in hepatocytes and transcription of genes involved in lipogenesis and β-oxidation (13). Additionally, mTOR has a central role in the regulation of cell cycle and initiation of transcription by translates the signaling for growth and proliferation. The administration of rapamycin (mTOR inhibitor) in patients with *PTEN* mutation has been found to be effective in reducing hamartomatous masses, lipomatous lesions, thymus hyperplasia with clinical recovery (8, 15,16). Hence, both hypoglycemia and uncontrolled cell proliferation in various tissues could be controlled with mTOR inhibition. With Sirolimus treatment, hypoglycemia of Case 2 has been controlled more succesfully.

Dysregulated PTEN-PI3K-AKT-mTOR signaling pathway may result in not only extensive tumor cell proliferation, but also deregulation of glucose metabolism. Increased glucose utilization is consistent with hyperactivation of the PI3K/Akt pathway being one of the key mediators of increased glucose utilization observed in many cancer cells (17). Kinross KM et al has developed a mice model with Pik3ca H1047R mutation. Pik3ca is the gene encoding the p110 a catalytic subunit of PI3K. In this model, a dramatic increase in body weight, which was associated with increased organ size, a reduction in blood glucose levels and undetectable insulin levels, were observed (18). In human, mosaic activating mutations in PI3K are known to cause segmental overgrowth. In a study which evaluated the metabolic phenotype of 22 patients with mosaic activating mutations affecting PI3K, three patients were found to have early onset, severe, nonketotic hypoglycemia (11).

With all of these findings, we concluded that dsyregulation of insuling signalling pathway affecting AKT2, PTEN, or PI3K could cause NkHH with syndromic features. Unexplained hypoglycemia mimicked that of hyperinsulinism with no detectable insulin should alert the physichian for possible insulin transducing defect. The effective treatment option of hyoglycemia in these patients could be the blocking of insulin signalling. The only known and experienced agent to impact this pathway is mTOR inhibitor Sirolimus. Until now, Sirolimus has been succesfully used for the severe, diffuse form of CHI (9). In our cases, we decided to give Sirolimus to improve hypoglycemia in a patient with *AKT2* mutation and another patient with PTEN tumoral hamartamatous syndrome caused by the inhibition of mTOR, which was the next step of PTEN/AKT signaling. In both patients hypoglycemia has been controlled with Sirolimus treatment.

The reported side effects of Sirolimus treatment were immunosuppression effects, oral mucositis, renal dysfunction, pneumonitis, increased serum aminotransferase levels, hepatitis, and dyslipidemia (9,19). We observed only mild leucocytosis in Case 1 without any additional symptoms.

In conclusion, NkHH is a very rare and significant disorder which provided some challanges in both diagnosis and treatment. Activating mutation of *AKT2* or *PTEN*, upstream from mTOR in insulin signalling, could lead to NkHH. Sirolimus treatment, as making mTOR inhibition, appeared to be effectively controlling the persistent hypoglycemia which could be a lifesaving tool for rare diseases caused by increased activation of insulin signalling.

Authorship Contribution:

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Figure 1: Case 1 with AKT2 mutation

Figure 2: Case 2 with PTEN mutation