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Neonatal Diabetes due to a Mutation in the Distal *PTF1A* Enhancer: A Case Report and Literature Review

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Abstract

Biallelic variants in the pancreas-specific transcription factor 1A (*PTF1A*) gene are a rare cause of permanent neonatal diabetes. We report a case of neonatal diabetes with unique clinical manifestations. The clinical diagnosis of the affected infant was confirmed by insufficient endocrine and exocrine pancreas activity; however, the pancreas was normal in imaging. Molecular analyses identified a novel homozygous single nucleotide variant (Chr10, g.23508441T > G), affecting a highly conserved nucleotide within a distal enhancer of the *PTF1A* gene. The literature review showed that most of these patients had IUGR and imaging evidence of pancreatic agenesis or hypoplasia. We suggest that pancreatic imaging and evaluation of exocrine pancreas function can help early confirmation of the diagnosis in patients with permanent neonatal diabetes.

Keywords: *PTF1A*, Pancreatic Agenesis, Cerebellar Hypoplasia, Neonatal Diabetes

1. Introduction

Neonatal Diabetes Mellitus (NDM) is defined as the onset of diabetes mellitus within the first six months of life (1), which is usually not autoimmune based. A genetic cause can be found for nearly more than half of the NDM patients (1). Mutations in *ABCC8* and *KCNJ11* are the most known common causes of NDM. Pancreatic and cerebellar agenesis are rare causes of neonatal diabetes. The pancreas-specific transcription factor 1A (*PTF1A*) gene encodes a transcription factor that plays a critical role in the early pancreas and cerebellar development (2). Mutation in *PTF1A* can impair this process and result in NDM. These patients show phenotypic variability, such as different ages for diabetes onset or involvement of cerebellum (2, 3).

Biallelic truncating variants in *PTF1A* have been reported in patients with pancreatic and cerebellar agenesis, whereas variants in the distal pancreatic-specific enhancer of the gene may cause isolated pancreatic agenesis (2). Here, we investigated the clinical features and genetic causes of NDM. We also reviewed clinical manifestations of previously reported cases of NDM caused by *PTF1A* mutation.

2. Case Presentation

The female neonate from a consanguineous Iranian family was presented during the first month of life with permanent neonatal diabetes. The pregnancy was uneventful. Fetal ultrasonography was normal. She was born with a gestational age of 39 weeks by cesarean section due to an abnormal non-stress test, suspicious of fetal distress. At birth, she passed meconium, and her Apgar score was standard. Her parents were first cousins; her grandmother was a known case of adulthood type 2 diabetes mellitus.

At birth, the body measurements were as follows: birth weight 1,800 g, length 43 cm, and head circumference 33 cm. Physical examination was normal, with no skeletal deformity or facial dysmorphism. She was admitted due to intrauterine growth retardation (IUGR). The hospital

course was complicated by pneumonia and sepsis, and she was admitted to Neonatal Intensive Care Unit (NICU) for 21 days. High blood sugar levels (400 mg/dL) were recorded since the 17th day of life but were thought to be "stress hyperglycemia". She received a few doses of regular insulin and was discharged at 21 days of age in good condition, without the maintenance of the insulin.

She was admitted again on the 53rd day of age because of chronic diarrhea and direct hyperbilirubinemia. Total and direct bilirubin levels were 9.7 mg/dL and 3 mg/dL, respectively. During this admission, the patient had repeated episodes of hyperglycemia. Therefore, regular and NPH insulins were started. Laboratory investigations showed fat malabsorption. Stool exam showed increased excretion of neutral fat (> 60 drops of neutral fat and > 100 drops of fatty acid in each microscopic field). Fecal elastase level was lower than 50 mg/gr (normal 200 - 500 mg/gr). Ophthalmoscopic exam and thyroid and renal function tests were normal. Liver enzymes were mildly elevated (ALT 72 u/L, AST 64 u/L), but this increase was transient, and the enzyme levels returned to the normal range. She had hypoproteinemia and hypoalbuminemia, with a total protein of 3 gr% and albumin of 2.5 gr%. Other laboratory tests were normal, including serum ammonia, PT and INR, 25-hydroxyvitamin D, amylase, and lipase.

Ultrasonographic study of the abdomen showed a normal-sized pancreas (length 50 mm and thickness 5 - 8 mm). magnetic resonance imaging (MRI) of the brain was completely normal without cerebellar involvement. Anti-islet cell antibody was negative, anti-glutamic acid decarboxylase (GAD) antibody was borderline (13.6 units/mL, normal range < 10 units/mL) and anti-insulin antibody was elevated (60 unit/mL, normal < 10 units/mL). Antibody measurements were performed a few months after starting insulin therapy, and high anti-insulin antibody levels can be secondary to exogenous insulin. The patient was discharged home in good general condition.

Treatment continued with NPH and regular insulin and exogenous pancreatic enzymes (creon, Solvay Healthcare, Hannover, Germany). At 18 months of age, the insulin regimen was switched to analog insulin, Levemir, and Apidra, with a dose of about 1unit/kg/day, creon was continued.

During the last visit at 23 months, the patient's body measurements were as follows: weight 11.6 kg (38th percentile), length 82.7cm (22nd percentile), with normal development and normal neurological and general physical examination.

2.1. Genetic Analysis

Genomic DNA was isolated from the peripheral blood of the patient. Sequencing the coding regions of *ABCC8*, *KCNJ11*, *INS*, and *EIF2AK3* genes did not identify a pathogenic variant. *PTF1A* gene sequencing covered the coding exons and distal enhancer (~ 400 bp sequence located 25 kb downstream the gene) using Sanger sequencing, as previously described (4). A single homozygous nucleotide variant (Chr10, g.23508441T > G) affects a highly conserved nucleotide within a distal enhancer of the *PTF1A* gene. Variants in this enhancer region mutation of a new distal developmental enhancer of *PTF1A* are a common cause of isolated pancreatic agenesis in humans (4). Specifically, two adjacent variants, g.23508437A > G and 23508446A > C have been previously identified in patients with isolated pancreatic agenesis (4). Tutak et al. reported an infant with neonatal diabetes and cerebellar agenesis who died at 42 days of age, but his mutation was not studied. Both of his parents were heterozygous for frameshift mutation (G240fsX276) in the *PTF1A* gene (5).

3. Discussion

We report a case of permanent neonatal diabetes, resulting from a homozygous *PTF1A* variant. Initial documented high blood glucose level was in the 1st month of life, which persisted until the last visit at 23 months old. She had pancreatic exocrine insufficiency requiring exogenous enzyme replacement; however, the pancreas was normal in ultrasonography. She did not have any neurological manifestation, and the brain MRI was normal.

3.1. Review of Previously Reported Cases

We reviewed the clinical features of previously 18 published cases (including our case) of NDM with a *PTF1A* variant. Table 1 shows the historical parameters of reported cases, and Table 2 compares their clinical and Paraclinical findings. Most cases were from the Middle East. Six cases (33%) were products of preterm deliveries (gestational age < 37 weeks) (3, 6-8), and the lowest gestational age was 31 weeks (7). The preterm birth did not affect clinical manifestations or prognosis. All except three of these cases had low birth weight (over 80%), probably because insulin has a significant role in fetal growth. The onset of hyperglycemia and initiation of insulin therapy in 14 patients were within

the first month of life (78%), which can be due to the effect of *PTF1A* variants on the fetal development of the pancreas. The presentation of hyperglycemia in one patient was as late as mid-childhood (9.5 years). A significant number of patients (44%) had failed to thrive (FTT), which was apparently due to malabsorption and not diabetes or neurological involvement. The growth of patients without malabsorption was within normal ranges (3, 6). The development of 10 patients (for which data was available) was normal. Five patients (28%) with high glucose levels immediately after birth died in early infancy (5, 6, 8, 9), so no clear correlation was identified between the age of onset and death. Little information was available regarding brain development. In one of the previously reported cases (6) and our patient had a normal brain MRI. However, semi-lobar holoprosencephaly was reported in one patient (9).

In 15 (out of 18) cases, pancreas imaging was performed. The pancreas was not detected (2, 6, 8-10) in eight cases, the pancreas was hypoplastic in six subjects, and the pancreas was normal in our patient, whereas the endocrine and exocrine functions were abnormal. These results may suggest that *PTF1A* variants can cause pancreas function abnormality, even without a significant structural defect.

Based on our review, PNDM is a genetically heterogeneous disorder due to mutations in 23 different genes described to date: *KCNJ11*, *ABCC8*, *FOXP3*, *GCK*, *PDX1*, *PTF1A*, *EIF2AK3*, *SLC2A2*, *GATA6*, *GATA4*, *SLC19A2*, *WFS1*, *NEUROD1*, *NEUROG3*, *RFX6*, *LRBA*, *NKX2-2*, *MNX1*, *IER3IP1*, *INS*, *STAT3*, *GLIS3*, and *HNF1B*. However, mutations in the genes encoding the ATP-sensitive potassium channel (KATP) subunits, *KCNJ11* (Kir6.2), *ABCC8* sulphonylurea receptor 1 (SUR1), and INS (insulin) compromise insulin secretion by affecting the mechanisms involved in insulin secretion. Also, previous functional analyses showed that a homozygous mutation could distrust enhancer activity and is likely to decrease in *PTF1A* expression during pancreatic development. This result confirms a diagnosis of neonatal diabetes and exocrine pancreatic insufficiency due to a recessive *PTF1A* mutation (11, 12). Therefore, we evaluated sequencing the coding regions of *ABCC8*, *KCNJ11*, *INS*, and *EIF2AK3* genes and also the *PTF1A* gene in our study. We report a case of permanent neonatal diabetes, associated with a homozygous *PTF1A* variant.

3.2. Limitations

There were some limitations in our study. Due to financial and time constraints, we evaluated sequencing of only coding regions of the other four genes (*ABCC8*, *KCNJ11*, *INS*, *EIF2AK3*), while other genes such as *PNDM*: *PDX1*, *RFX6*, *GATA4*, *GATA6*, *GLIS3*, *NEUROD1*, *PAX6*, *MNX1*, *NKX2-2*, *GCK*, etc. that might have been related to permanent neonatal diabetes were not screened. The screening of them is suggested in another study.

3.3. Conclusions

Our study showed that there is an association between the identified *PTF1A* gene variant and permanent neonatal diabetes. Since most previously reported patients had IUGR, and imaging evidence of pancreatic agenesis or hypoplasia, NDM due to *PTF1A* variants should be considered in the differential diagnosis of neonatal diabetes (especially with the onset in the first month of life). We suggest that pancreatic imaging and evaluation of exocrine pancreas function can help early confirm the diagnosis in these patients. Our results expand the clinical phenotype associated with *PTF1A* enhancer mutations and highlight the importance of genetic testing and clinical follow-up studies to characterize rare genetic subtypes of diabetes. These results can be applied in screening and genetic counseling, especially in Middle Eastern populations.

Footnotes

Authors' Contribution: Study concept and design: H.M. and A.H.; Acquisition of data: H.M., A.H., and F.M.; Analysis and interpretation of data: H.M. and H.I.; drafting of the manuscript: H.M., A.A. and H.I.; critical revision of the manuscript for important intellectual content: A.A. and A.H.; statistical analysis: H.M. and F.M.; Administrative, technical, and material support: F.M.; Study supervision: H.M. and A.A.

Conflict of Interests: All authors declare that there is no conflict of interest.

Ethical Approval: For providing this case report , informed consents was taken from patients' parents

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Informed Consent: Oral informed consent was taken from patients' parents for this case report.

Table 1. Significant Historical Parameters of Reported Cases, NA: Not Available

No.	Reference No.	Gestatio-l Age (w)	Sex	Birth Weight (g)	Age at Presentation	Consanguinity	Development	Ethnic or Country of Origin
1	(6)	38	M	1,980	1 day	Yes	Normal	Saudi Arabia
2	(6)	37	F	2,000	1 day	Yes	Normal	Saudi Arabia
3	(6)	34	M	1,275	1 day	Yes	Normal	Kuwait
4	(6)	36	F	1,400	8 days	Yes	Normal	Kuwait
5	(2)	37	M	1,935	7 days	No	-	European
6	(9)	38	F	1,100	1 day	No	-	European
7	(3)	35	F	1,450	10 days	Yes	Normal	Half-Turkish Half-Kurdish
8	(3)	38	F	2,600	9 years	Yes	Normal	Half-Turkish; Half-Kurdish
9	(7)	32	?	1,200	3 weeks	Yes	-	Turkish
10	(7)	39	?	2,400	10 weeks	Yes	-	Turkish
11	(7)	31	?	1,500	1 week	Yes	Delayed	Turkish
12	(10)	39	M	2,800	4 weeks	Yes	Normal	-
13	(10)	39	F	2400	3 weeks	Yes	Normal	-
14	(10)	38	F	3,000	78 weeks	Yes	Normal	-
15	(10)	38	M	2,300	2 weeks	Yes	Normal	-
16	(5)	39	M	1,660	2 days	Yes	Delayed	Turkish
17	(8)	36	M	< 3rd percentile	2 months	Yes	Delayed	Saudi Arabia
18	This paper	39	F	1,800	17 days	Yes	Normal	Iranian

Table 2. Significant Clinical and Paraclinical Findings in Reported Cases.

No.	Reference No.	Facial Dysmorphism	Failure to Thrive	Malabsorption	Neurological Involvement	Imaging of Pancreas	Imaging of Brain	Outcome	Mutation
1	(6)	-	Yes	Yes	No	Not identified pancreas in ultrasonography	Not performed brain MRI	Alive	c.571C > A
2	(6)	-	No	No	No	Not identified pancreas in ultrasonography	Not performed brain MRI	Alive	c.571C > A
3	(6)	-	Yes	Yes	No	-	Not performed brain MRI	Died at 12 weeks	
4	(6)	-	Yes	Yes	No	-	Normal brain MRI	Alive	
5	(2)	-	Yes	Yes	-	Pancreas could not be identified in ultrasonography	-	Alive	Compound hetero g.23508442A > G and…
6	(9)	Yes	Yes	Yes	Yes	Absence of pancreas and gallbladder in abdomen	Brain MRI semilobar holoprosencephaly	Died at 12weeks	
7	(3)	-	Yes	Yes	Yes	Severe pancreatic hypoplasia of head, tail and body were nearly undetectable	-	Alive	Homozygous for g.23508437A > G
8	(3)	-	No	No	No	Pancreatic hypoplasia	Normal brain MRI	Alive	Homozygous for g.23508437A > G
9	(7)	-	-	-	-	Pancreatic hypoplasia	-	Alive	Homozygous for g.23508365A > G
10	(7)	-	-	-	-	Pancreatic hypoplasia	-	Alive	Homozygous for g.23508437A > G
11	(7)	-	-	-	Yes	Pancreatic hypoplasia	-	Alive	Homozygous for g.23508437A > G
12	(10)	-	-	Yes	-	Pancreatic agenesis	-	-	
13	(10)	-	-	Yes	-	Pancreatic agenesis	-	-	
14	(10)	-	-	Yes	-	Pancreatic hypoplasia	-	-	
15	(10)	-	-	Yes	-	Pancreatic agenesis	-	-	
16	(5)	Yes	Yes	-	Yes	-	Absence of Cerebellum	Died at 42 days	Not checked
17	(8)	Yes	Yes	-	Yes	Absence of pancreas	Cerebellar agenesis	4 months	c.437-460del
18	This paper	No	No	Yes	No	Normal	Normal	Alive	g.2350844IT > G

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