LATE DIAGNOSIS OF GANGLIONEUROBLASTOMA IN AN INFANT

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Abstract

The neuroendocrine masses that synthesize vasoactive intestinal peptide (VIP) are named VIPomas.Watery diarrhea, hypokalemia, achlorhydria syndrome (WDHA) occurs frequently in adults with pancreatic tumors. WDHA syndrome in children is mainly induces by VIPomas localized in mediastinum or retroperitoneum. Chronic diarrhea in infants is a common condition for addressability to pediatric gastroenterologists. The causes are multiple and the delay in reaching the final diagnosis can lead to fatal complications. The authors present an infant with recurrent watery diarrhea, subocclusion manifestations, hyperchloremic metabolic acidosis and hypokalemia triggered by a retroperitoneal VIPoma that was diagnosed by abdominal ultrasound and tomography. Laboratory investigations indicated an elevated VIP serum level. Tumor excision restored the normal stool consistency, corrected the imbalance of serum electrolytes and normalized VIP level. The diagnosis of N-MYC negative ganglioneuroblastoma was confirmed by immune-histochemical assessment. This paper describes the clinical and histo-genetic aspects of this rare clinical condition.

Key words: infant, diarrhea, vasoactive intestinal polypeptide, ganglioneuroblastoma

Introduction

Verner and Morrison reported in 1958 chronic watery diarrhea with hypokalemia triggered by pancreatic masses.[1] The patients with Verner-Morrison syndrome usually associate reduced gastric secretion or achlorhydria and severe diarrhea similar to that induced by Vibrio cholerae.[2] For this reason, the syndrome was named ”pancreatic cholera”.[2] Some authors proposed the acronym WDHA for watery diarrhea, hypokalemia and achlorhydria[3], albeit the term WDHHA (watery diarrhea, hypokalemia, hypochlorhydria and acidosis) would be more appropriate accounting the loss of bicarbonate.[3] Several publications of case series have described the concomitance of recurrent watery diarrhea with pancreatic masses.[4, 5, 6] This syndrome is mainly associated with pancreatic tumors in adult patients. Only in rare cases it can be triggered by extra-pancreatic tumors as retroperitoneal histiocytoma, adrenal pheochromocytoma, bronchogenic carcinoma or medullary thyroid carcinoma.[7]

In infants, it is extremely rare for a vasoactive intestinal polypeptide (VIP) synthesizing mass to belong to the pancreas. More often, WDHA syndrome in children is determined by VIPomas originating in the mediastinum and retroperitoneum.[5] Although being reported in childhood, pancreatic non–beta-cell hyperplasia is very scarce among pediatric patients.[8, 9] There were only a few case series published regarding this topic. [8, 9]

A vast array of gastrointestinal disorders may induce recurrent diarrhea in pediatric patients. In a minority of cases, diarrhea is a consequence of active intraluminal liquid outpouring, known as secretory diarrhea. Due to important diagnostic implications, the identification of such pathologic cases comes as a necessity. Recurrent secretory diarrhea without an obvious intestinal cause may be due to an occult tumor mass that produces VIP.

In this paper, the authors describe an infant diagnosed with electrolytes imbalance–low kalium level and chronic secretory diarrhea caused by a retroperitoneal VIP producing tumor which was immune-histochemically confirmed to be a N-MYC negative ganglioneuroblastoma. Tumor excision restored the normal stool consistency, corrected the imbalance of serum electrolytes and normalized VIP level. The authors obtained the written informed consent from the parents of the child to publish this case report.

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Case presentation

An 11 months old female patient was referred to our hospital with a history of watery diarrhea, malnutrition and important abdominal distension, without vomiting. For one month, the infant presented recurrent diarrhea with seven to eight watery stools daily without pathological products (mucus or blood).

The child originates from a rural environment, the mother being aged 42 years old and the father 40 years old. No relevant familial history for chronic diseases was noted. The female child was the eighth born infant by natural birth, with the gestational age of 38 weeks, weighting 3100 grams and being given an APGAR score of nine points. The child was vaccinated according to the national program. She has been breastfed for one month and was then switched to formula. Also, complementary feeding has been properly initiated at the age of four months. The infant has no significant medical history. During the first ten months of life, she presented a normal intestinal function. However, at the age of ten months, watery diarrhea occured, without fever or vomiting.

Before hospital admission, during four weeks of outpatient investigations the diagnosis of cow's milk protein allergy was established. Dairy were excluded and the infant started a semi-elemental diet containing hydrolyzed proteins without lactose, but the trial of cow's milk exclusion had been unsuccessful. Empiric administration of Furazolidon for an alleged parasitosis was useless. The infant received oral rehydration solution repeatedly, but the diarrhea persisted.

Upon admission, the infant's height was 70 cm (5th percentile according to age) and the weight was eight kg (25th percentile according to age). Physical examination indicated normal blood pressure (80/55 mmHg) and regular heart rate of 85 beats/minute.The infant presented facial flushing, important abdominal distension without liver or spleen enlargement or abdominal mass detected through palpation. Laboratory tests showed mild hypokalemia and metabolic acidosis. There were no abnormal results of routine biochemical investigations and blood cell count.We ruled out infectious enterocolitis, intestinal parasitosis, cow's milk protein allergy, celiac disease and exocrine pancreas insufficiency/mucoviscidosis. Diarrhea persisted even after implementation of modular, elemental amino-acid based diet.

Due to the persistence of an important abdominal distension, X rays examination of the abdomen was performed showing enlarged small and large intestine with air-fluid levels without a lumen-occluding mass (Figure 1).

An abdominal ultrasound, followed by abdominal computed tomography (CT) scan showed a calcified tumor with a size of six/four cm in the retroperitoneal left lateral area, anterior to the L1-L4 lumbar spine and aorta, without contact with the left kidney. The pancreas did not present any abnormalities (figure 2).

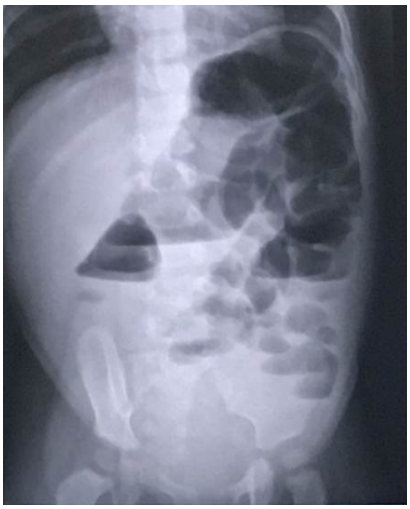


Figure 1: Radiological imaging showing enlarged small and large intestine with air-fluid levels without a lumen-occluding mass.

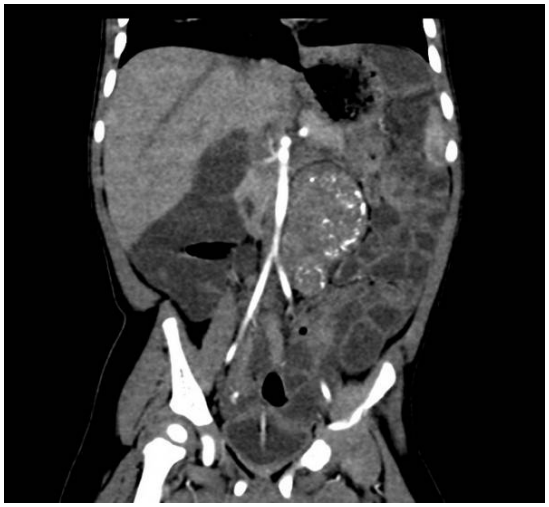


Figure 2: CT scan depiction of the retroperitoneal tumor with calcifications.

Additional endocrinological evaluation was applied in order to ensure an accurate diagnosis. The thyroid did not show any signs of disfunctionality. Serum hormone levels was assessed and the results showed high values of VIP =180 pmol/l (normal range <30 pmol/l). In addition, the 24 urinary vanillylmandelic acid levels were normal as well as those of the homovanillic acid. However, neuron specific enolase showed elevated levels - 25,77 ng/ml (normal range <16,3 ng/ml), raising the suspicion of a possible VIP secreting ganglioneuroblastoma.

The infant was referred to the Department of Pediatric Surgery. Surgical exploration showed an encapsulated retroperitoneal tumor measuring of six/four cm, anterior to the L1-L4 lumbar spine and aorta, without contact with the left kidney. The aspect of the pancreas did not present any notable changes. Tumor excision was then performed.
Figure 3 shows the macroscopic aspects of the tumor.

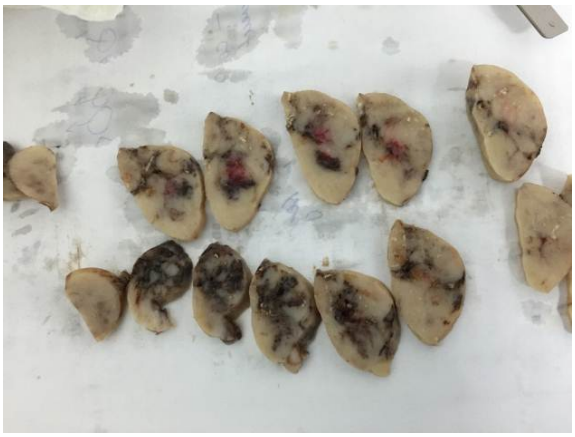
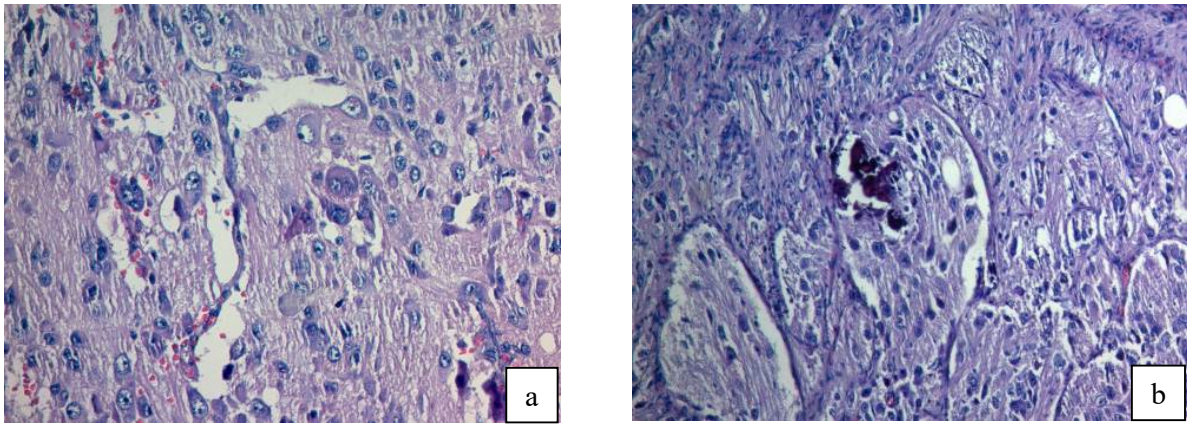


Figure 3: Macroscopic aspects of the tumor reveal a brown color, an elastic consistency and visible calcifications on each section.

Histological examination of the tumor established the diagnosis of ganglioneuroblastoma intermixed with stroma rich (Shimada Classification [10]), International Neuroblastoma Staging System (INSS) [10] stage two localized tumor, with incomplete gross removal, positive lymph nodes, MKI (mitosis-karyorrhexis index) low < 100. The tumor exhibited a lobular/solid growth pattern. The tumor cells were characterized by an abundant basophilic cytoplasm, with large, round to ovoid nuclei. Prominent nucleoli were noted along with alternative areas of euchromatin and heterochromatin, slightly mimicking a "salt and pepper appearance". Other tumor cells were characterized by eccentric nuclei, distinct cell borders and a rich, eosinophilic cytoplasm. A low amount of hemorrhage was present amongst the tumor cells along with moderate areas of dystrophic calcification. Figure 4a and Figure 4b reveal the microscopic aspect of the tumor on Haemotoxylin and Eosin (H&E) stained specimens.



Figures 4a and 4b: Depiction of the enlarged nuclei of tumor cells exhibiting visible nucleoli and the rich, eosinophilic cytoplasm. Amongst tumor cells, slight areas of hemorrhage have been observed (4a). Note the lobular/solid growth pattern of the tumor with the presence of moderate areas of dystrophic calcification (4b). H&E stained specimen x40 magnification.

In order to establish the final diagnosis and exclude other similar lesions, additional immunohistochemical analysis was applied using the following panel of markers: NSE, Synaptophysin, NF 200, S100, GFAP, Chromogranin and CD99. Vimentin was used as an internal control marker and exhibited a positive stromal reaction. NSE, Synaptophysin and NF 200 exhibited a moderate to strong reaction in the tumor cells. The pattern of reaction was cytoplasmic and presented a diffuse, heterogeneous distribution. S100 and GFAP presented a strong immunohistochemical reaction in the ganglion cells and in stroma. Additionally, S100 was positive in the cytoplasm of Schwann cells. Neuroblasts proved to be isolately positive for Chromogranin. CD99 was evaluated as negative overall. The immunohistochemical features of the tumor are revealed in Figures 5a, b, c and d.

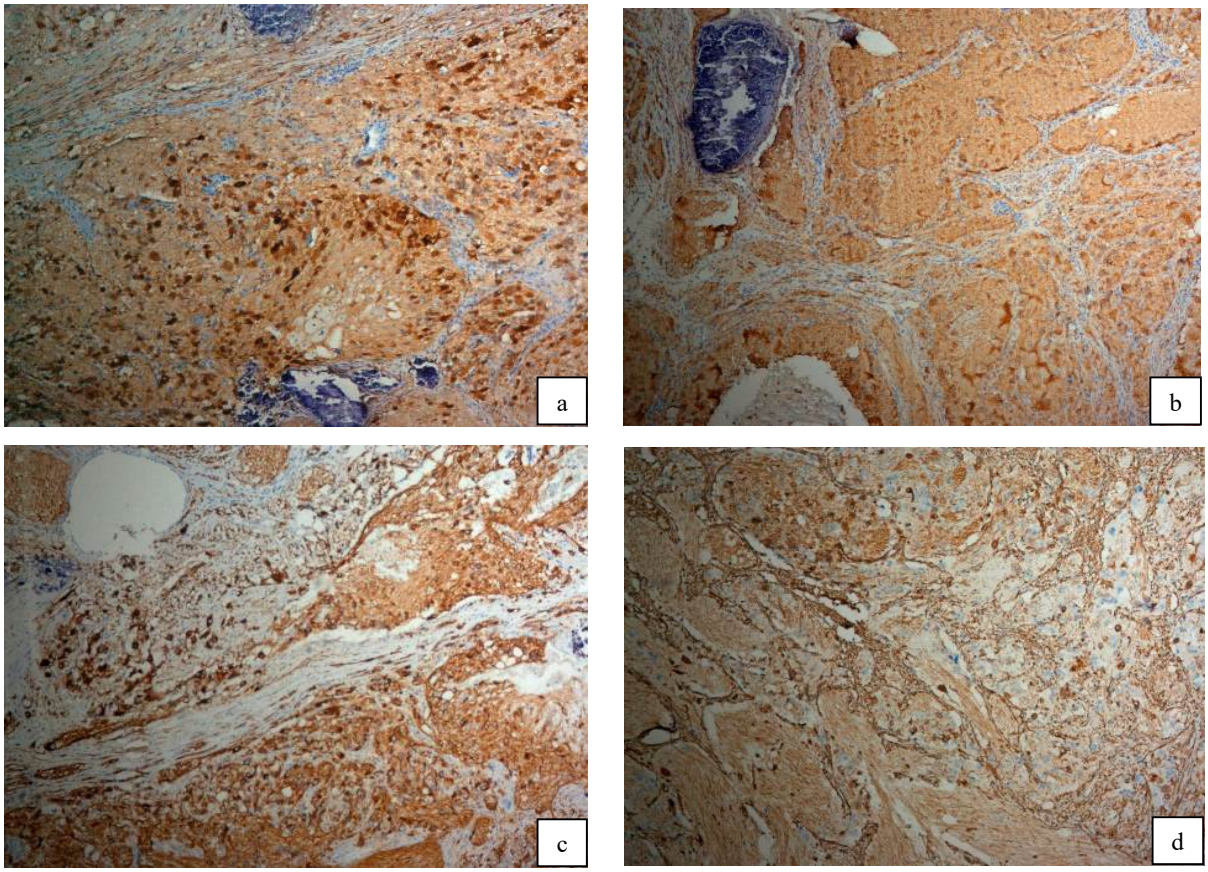


Figure 5a, 5b, 5c, 5d: Protein S100 exhibited a strong, positive reaction in both Schwann cells and ganglion cells (5a). NSE was intensely expressed in the tumor cells, with a cytoplasmic pattern of reaction (5b). Note the intense, diffuse, rather homogeneous expression for NF 200 in the tumor cells (5c). GFAP exhibited a moderate to strong positive expression in the ganglion cells (5d). Specimen x10 magnification.

Further molecular biology tests were performed. The N-MYC gene status was assessed by fluorescence in situ hybridization (FISH) technique and the result was negative.

The evolution after surgical tumoral excision was favorable. The stool consistency restored to normal and laboratory test showed correction of serum kalium levels. Subsequently, there was no diarrhea recurrence after the follow up period. The plasma concentration of the vasoactive intestinal polypeptide remained at low levels on regular measurements following surgical intervention.

Discussions

Although rare, Werner-Morrison Syndrom is a well-known clinical and pathological condition induced mainly by VIP-secreting masses.[1] The majority of VIPomas diagnosed in children are ganglioneuromas or ganglioneuroblastomas, developed from the neural crest of the adrenal medulla or sympathetic ganglia. Ganglioneuromas are benign tumors characterized by well differentiated structure, while ganglioneuroblastomas display variable degree of differentiation and have an uncertain prognosis.[11] These tumors may originate everywhere the sympathetic nerve tissue exists. The adrenal glands (35%), retroperitoneal ganglia (30-35%), posterior mediastinum (20%), pelvis (2-3%) and head and neck (1-5%) represents the most frequent localizations. More rarely, these tumors are found in anterior mediastinum, thymus, lung or kidney. [12]

Ganglioneuromas, ganglioneuroblastomas and neuroblastomas differ from one another according to the cells maturation stages. Being composed of mature cells, ganglioneuromas are considered benign tumors. Ganglioneuroblastomas are more immature masses, bearing a higher potential of invasion. These tumors usually develop in infants and toddlers, the medium age at onset being around two years. Patients diagnosed with ganglioneuroblastomas present a relatively good prognosis considering that these masses may spontaneously regress or maturate into ganglioneuromas. Regression develops in 1-2% of cases and the reason is uncertain.[11]

Initially, a hypothesis has been released in the 1960s, supporting the idea that watery diarrhea was provoked by synthesis of high level of catecholamine, a well-known fact that was associated with neural crest masses.[13] Later, in the 1970s, researchers have proved that these tumors also secreted VIP.[14] VIP is characterized by a 3381 molecular

weight and consist of a sequence of 28 amino acids, appertaining to the secretin-glucagon class.[15] In physiological situations, VIP is synthesized at the level of central nervous system and in the neural plexus of the digestive, urogenital and respiratory tract, acting as a neurotransmitter. [15]

VIP overexpression causes diarrhea and its receptors overexpression may induce malignant proliferation. At the gastrointestinal level, VIP leads to relaxation in smooth muscle cells of vascular and nonvascular structures and regulates intraluminal transport of electrolytes and water. Food consumption triggers intestinal dilatation and secondary VIP releasing. Intestinal cyclic adenosine monophosphate (cAMP) synthesis is stimulated by VIP hormone, promoting important intraluminal secretion of kalium along with other electrolytes and water. The patients with neuroendocrine masses that synthesize high levels of VIP hormone experience significant watery diarrhea with secondary dehydration, failure to thrive and facial flushing.[16]

VIP concentration was raised in the hereby presented case report. Moreover, hypokalaemia was documented. About 90% of children with a neuroblastoma will present an excessive homovanillic and vanillylmandelic acid production. If vanillylmandelic acid along with other catecholamine levels are within normal ranges, the diagnosis of neuroblastoma is less probable; however, this diagnosis cannot be excluded.[17] The neuroendocrine tumors do not synthesize catecholamines permanently. Therefore, the secreted hormones and their metabolites may have unsteady peaks with fluctuating levels in the blood and urine.[18] Neuron specific enolase (NSE) is a well-represented isoenzyme synthesized in neuroendocrine tumors and neurons, described as a 78 kD gamma-homodimer.[19] NSE concentration in other organs, with the exception of erythrocytes, are non-detectable.[19] Having a high specificity for the above mentioned organs and tissues, NSE serum or cerebrospinal levels are increased in almost all disorders that associate neuronal destruction. Also, patients with tumors derived from the neural crest usually present elevated NSE levels. In almost 70% of cases with small cell lung carcinoma high NSE levels were detected at the onset of the disease.[20] NSE may also present high serum levels in certain neuroendocrine malignancies as carcinoid tumors (66% of patients), islet cell tumors (up to 40% of patients) or neuroblastoma (with unknown prevalence of patients that associate elevated serum levels). [20,21]

In this case, vanillylmandelic and homovanillic 24 urinary levels were normal, but neuron specific enolase serum level was elevated, indicating, along with the elevated VIP serum levels, the diagnosis of VIP producing ganglioneuroblastoma that was further certified by histopathological examination and imunohistochemical staining.

According to the International Neuroblastoma Risk Group (INRG) classification[22], this patient was included into the low risk group based on histological criteria, degree of differentiation, age and N-MYC negative test.

In this case, the initial suspicion was referring to a gastrointestinal condition. This fact delayed the correct diagnosis. The infant underwent four weeks of different medical explorations as an outpatient and the period starting from hospital admission to diagnosis was of two weeks time. Empiric administrations of various medications and special diets for unproved digestive disorders were useless. Early recognition of secretory diarrhea would have facilitated a rapid diagnosis and would have avoided unnecessary tests.

In certain cases infectious gastroenteritis (viral or bacterial) may induce intraluminal secretion. But chronic secretory diarrhea due to infectious causes is scarce. There are some rare genetic conditions that occur in the neonatal period and are characterized by the presence of secretory diarrhea due to electrolyte transport inherited errors as congenital sodium or chloride diarrhea. These are easily ruled out when the persistent watery diarrhea occurs later in infancy.

In this case, the symptoms occurred at the age of 10 months old, allowing us to initially rule out the diagnosis of congenital electrolyte transport defects.

Later in childhood, secretory diarrhea may appear in context of other gastrointestinal conditions as short bowel syndrome or inflammatory bowel diseases. However, in these disorders, the intestinal injury as cause for persistent diarrhea is easily recognized.

Conclusions

Secretory diarrhea may be the only inaugural symptom of a VIPoma and consequently appropriate explorations and treatment strategies must be initiated for early diagnosis.

This report describes an atypical case of VIP-secreting ganglioneuroblastoma in an infant that presented diarrhea as the main clinical sign. In case of a child with chronic watery diarrhea of unknown etiology it is recommended to carry out both gastrointestinal explorations and abdominal imagistic tests in order to establish whether or not a tumor is the cause of intraluminal secretion.

From a morphological perspective, the examined tumor presented particular features that made the differential diagnosis a difficult step. Neuroblasts lacked the specific pseudo rosettes layout and cystic degeneration was absent. We did not notice the presence of neuromelanin pigment inclusions, that are documented in literature as being rare.

Ganglioneuroblastomas are controversial clinical and pathological entities, the morphologic and immunohistochemical distinction between ganglioneuromas, neuroblastomas and Schwannomas with neuroblastoma-like features being regarded as a difficult diagnostic approach. The final diagnosis of such rare pathologic cases is complex and mostly based on the integration of the clinical presentation of the patient, with imaging examination, histopathological routine analysis and additional immunohistochemical evaluation of the specimens.

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