CASE REPORTS

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Congenital thrombocytopenia with nephritis – The first case of MYH9 related disorder in Serbia

Kongenitalna trombocitopenija sa nefritisom – prvi bolesnik sa MYH9 poremećajem u Srbiji

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Abstract

Introduction. The group of autosomal dominant disorders – Epstein syndrome, Sebastian syndrome, Fechthner syndrome and May-Hegglin anomaly – are characterised by thrombocytopenia with giant platelets, inclusion bodies in granulocytes and variable levels of deafness, disturbances of vision and renal function impairment. A common genetic background of these disorders are mutations in MYH9 gene, coding for the nonmuscle myosin heavy chain IIA. Differential diagnosis is important for the adequate treatment strategy. The aim of this case report was to present a patient with MYH9 disorder in Serbia. **Case report.** A 16-year-old boy was referred to our hospital with the diagnosis of resistant immune thrombocytopenia for splenectomy. Thrombocytopenia was incidentally discovered at the age of five. The treatment with corticosteroids on several occasions was unsuccessful. Although the platelet count was below 10×10^9/L, there were no bleeding symptoms. Besides thrombocytopenia with giant platelets, on admission the patient also suffered sensorineuronal hearing loss and proteinuria. The diagnosis was confirmed with immunofluorescence and genetic analyses. **Conclusion.** Early recognition of MYH9-related diseases is essential to avoid unnecessary and potentially harmful treatments for misdiagnosed immune thrombocytopenia, and also for timely and proper therapy in attempt to delay end-stage renal failure and improve quality of life.

Key words:
thrombocytopenia; nephritis hereditary; myosin heavy chains; diagnosis, serbia.

Apstrakt

Uvod. Grupu autozomno dominantnih poremećaja – Epsteinov sindrom, Sebastianov sindrom, Fechthnerov sindrom i May-Hegglinovu anomaliju – odlikuju trombocitopenija sa džinovskim trombocitima, inkluzije u granulocitima, kao i različita zastupljenost gluvoće, poremećaja vida i funkcije bubrega. Genetska osnova ovih sindroma su mutacije u genu za teški lanac nemišićnog miozina IIA, a za ovu grupu sindroma predložen je naziv bolesti vezane za MYH9. Diferencijalna dijagnoza prema trombocitopenijama druge etiologije je značajna zbog pravilnog izbora terapijskih postupaka. Cilj rada bio je prikaz bolesnika sa MYH9 poremećajem u Srbiji. **Prikaz bolesnika.** Bolesnik, star 16 godina, sa dijagnozom rezistentne imunske trombocitopenije upućen je radi daljeg lečenja splenektomijom. Trombocitopenija je otkrivena u petoj godini života rutinskim pregledom krvne slike. U više navrata bolesnik je lečen kortikosteroidima ali bez povoljnog terapijskog odgovora. Iako je broj trombocita najčešće bio manji od 10×10^9/L, nisu se javljali simptomi krvarenja. Pored trombocitopenije sa džinovskim trombocitima, na prijemu su nađeni senzorineuralna gluvoća kao i proteinurija. Dijagnoza je potvrđena imunofluorescentnim nalazom i genetskom analizom. **Zaključak.** Pravovremeno prepoznavanje poremećaja mutacije MYH9 neophodno je kako bi se izbegli neadekvatni i potencijalno opasni načini lečenja koji se primenjuju kod imunske trombocitopenije. Takođe, odgovarajućom terapijom odlaže se razvoj terminalne bubrežne insuficijencije i poboljšava kvalitet života.

Ključne reči:
trombocitopenija; nefritis, nasledni; miozin, teški lanci; dijagnoza; srbija.

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Introduction

The group of autosomal dominant disorders formerly called Epstein (OMIM # 153650), Fechtner (OMIM # 153640), and Sebastian syndrome (OMIM # 605249) and May-Hegglin anomaly (OMIM # 155100) is characterized by thrombocytopenia with giant platelets and Döhle-like inclusion bodies in granulocytes. The diagnosis was established on the clinical grounds, assessing the involvement of kidney, inner ear or eye [1, 2]. A decade ago, it was recognized that these different entities have unique genetic background, with variable clinical expression, varying from mild macrothrombocytopenia with leukocyte inclusion bodies to a severe form complicated by hearing loss, cataract and renal failure [3–5]. Since all clinical features are the consequence of different mutations in MYH9 gene, the new term "MYH9 disorders" or "MYH9-related disease" (MYH9-RD) was proposed [4]. MYH9 is the gene encoding for the nonmuscle myosin heavy chain IIA (NMMHC-IIA), which is localized on chromosome 22q11-13 [5].

In this paper we presented the clinical and laboratory findings, and the course of the disease in a 16-year-old boy suffering from MYH9-RD, with clinical features previously classified as Epstein syndrome [1], which was misdiagnosed as immune thrombocytopenia in early childhood. To the best of our knowledge this is the first report on MYH9-RD in Serbia, as well as in the region of Southeastern Europe.

Case report

The boy was admitted to our hospital at the Department of Hematology for the first time at the age of 16 years for evaluation of thrombocytopenia and for assessment for splenectomy. Thrombocytopenia was discovered incidentally at the age of five, and he was treated with prednisolone on several occasions, with no response. Although the platelet count was most of the time below 10×10^9/L, the patient was almost free of bleeding symptoms. Surgical correction of hypospadia at the age of seven years took an uneventful course, without unexpected bleeding. At the time of admission, full blood count showed a very low platelets count of 4×10^9/L, with giant platelets on blood smear (Figure 1A), hemoglobin (Hgb) was 138 g/L, red blood cells (RBC) 4.08×10^{12}/L, mean corpusculer volume (MCV) 98.8 fl and white blood cells (WBC) 7.0×10^9/L. Bone marrow aspirate revealed normal cellularity, with small, hypolobulated megakaryocytes. Platelet kinetics, investigated with indium-111 labelled autologous platelets, showed significantly decreased platelet production whose life span was shortened to 3.6 days.

Urine analysis revealed microscopic hematuria and nephrotic range proteinuria, with 24-hour protein excretion of 5.5 g. Blood chemistry showed normal levels of serum protein, albumin and cholesterol. Serum urea and creatinine were 5.4 mmol/L and 77 μmol/L, respectively, and the estimated glomerular filtration rate (eGFR) was 134.4 mL/min per 1.73 m^2. Owing the risk of bleeding due to thrombocytopenia, renal biopsy was not done.

Findings of urine abnormalities prompted further investigations and a more detailed family history. Both patient parents were healthy; father's blood pressure, platelet count, GFR, urine sediment and 24-hour protein excretion all were normal. Audiometry showed high tone sensorineural deafness with hearing defects of more than 70 db in the range of high tone frequencies (2,000 and 4,000 Hz). Ophthalmologic examination gave normal findings. On the basis of thrombocytopenia with giant platelets, hearing defect and renal abnormalities, the clinical diagnosis of Epstein syndrome was made [2, 3].To further substantiate the Epstein syndrome diagnosis, peripheral blood smears were sent to a specialized laboratory [6]. Immunocytochemistry for NMMHC-IIA organization in neutrophils was reported as normal, and therefore, any further investigations were considered unnecessary. Treatment with an angiotensin-converting enzyme (ACE) inhibitor was started and hearing amplification was prescribed. At follow up appointment, two months later, 24-hour protein excretion was reduced to 3.5 g. Unfortunately, the patient stopped taking ACE inhibitor after two months and refused to use hearing amplifications. At the last regular follow-up visit, at his 16 years and 9 months of age (8 months after the first referral) his blood pressure (BP) was 140/80 mmHg, serum urea 4.6 mmol/L, creatinine 89 μmol/L (1.0 mg/dL), the estimated GFR 91.9 mL/min *per* 1.73 m^2, and proteinuria 4.37 g/24hr. The patient did not come to follow-up visits during the next three years. When we finally succeeded to contact him, he was 19 years and 8 months old, deaf and with end-stage renal disease (ESRD); eGFR was 11.2 mL/min *per* 1.73 m^2, serum urea and creatinine levels were 23 mmol/L and 726 μmol/L, respectively. Urinalysis confirmed hematuria and nephrotic range proteinuria (7g/24h). His platelet count remained low (4×10^9/L).

These findings convinced us more deeply in the diagnosis of Epstein syndrome. Reanalysis of our patient's peripheral blood smear was done at the Clinical Research Center of Nagoya Medical Center, Japan. Although granulocyte inclusion bodies were invisible on peripheral blood [7] smears (Figure 1A), immunofluorescence analysis revealed abnormal NMMHC-IIA localization in neutrophils (Figure 1B). After extraction of DNA from the remaining peripheral blood smear, MYH9 sequence analysis disclosed p.R702C missense mutation, finally confirming our clinical diagnosis (Figure 1C).

Discussion

The main manifestations of MYH9-RD, i.e. thrombocytopenia, giant platelets, and granulocyte inclusion bodies, are present at birth. In most cases thrombocytopenia is found incidentally, because the associated bleeding tendency is mild. Glomerulonephritis develops in 30–70% of patients with MYH9-RD, usually at mean age of 23 years and ESRD develops before the fourth decade of life in the majority of patients [8]. More than half of patients have bilateral or unilateral sensorineural hearing loss for high tones. Presenile cataract has the lowest incidence of all clinical MYH9-RD manifestations [9].

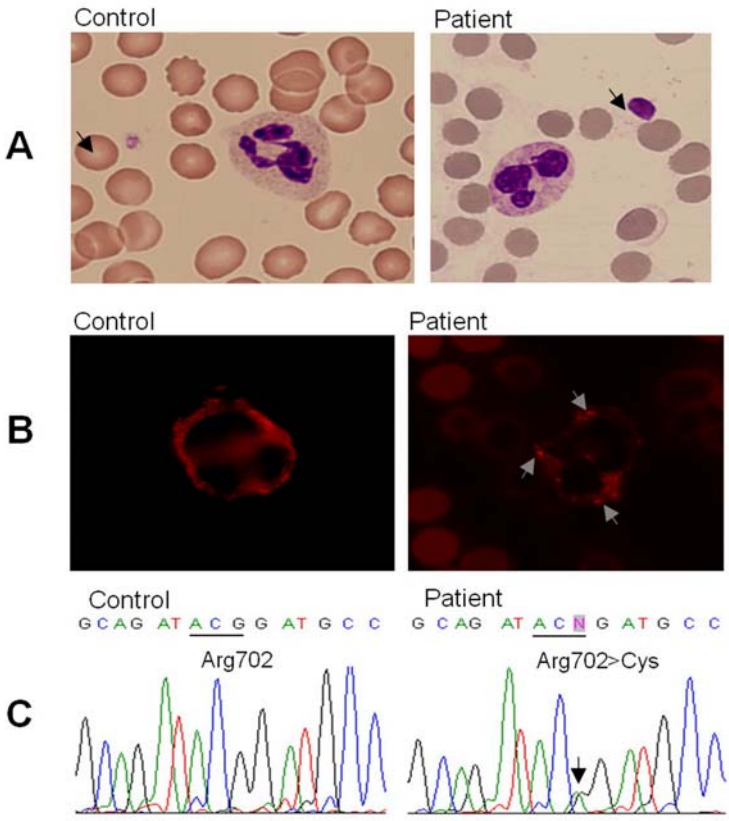

Fig. 1 – A. Giant platelet in the patient compared to that from the normal control; B. Abnormal NMMHC-IIA aggregations in the cytoplasm of neutrophils are indicated by arrows; C. Sequence electrophoregram showing c.2104C > T substitution resulting in p.R702C in the patient.

Up to date, more than 40 different mutations in the MYH9 gene have been identified. Most affected individuals have missense, nonsense or frame shift mutations in only 6 exon locations that have been found in 80% of affected families [9].

There is a genotype – phenotype correlation regarding the onset and severity of disease. Mutations in the motor domain of MYH9 are frequently associated with the development of nephritis and deafness, whereas mutations in the tail domain are associated with a lower risk (10%) of developing such impairments. Mutations at Arg702 produce glomerulopathy and deafness at a juvenile age [10]. Association of the p.R702C missense mutation with earlier progression to end-stage renal disease (ESRD) explains rapid deterioration of renal function in our patient as it was reported previously [9, 11, 12]. Sekine et al. [12] found that patients with this mutation over 15 years old developed ESRD between 15 and 20 years, each of them progressed to ESRD shortly after serum creatinine level exceeded 1.0 mg/dL. The above presented data show that our patient's clinical course followed this pattern: he reached ESRD within 3 years after his serum creatinine level rose to 1.0 mg/dL.

Although the clinical diagnosis of Epstein syndrome in our patient was made on initial examination, the molecular genetic confirmation was delayed because the initial immunocytochemistry diagnostics of granulocyte NMMHC-IIA localization, conducted by an outside research organization was negative [6]. We reevaluated its localization by the immunofluorescence analysis and found abnormal accumulation/aggregation [7] (Figure 1B). Subsequent DNA analysis revealed MYH9 p.R702C missense mutation in the patient. Although faint staining of inclusion bodies using May-Grünwald-Giemsa stains has the potential to hamper the diagnosis in patients with MYH9 head domain mutations such as at Ser96 and Arg702, immunofluorescence analysis can detect abnormal NMMHC-IIA localization. Thus, immunofluorescence analysis but not immunocytochemistry analysis facilitates the diagnosis of MYH9-RD.

The absence of clinical and laboratory abnormalities in the parents suggests *de novo* origin of the mutation in our patient. MYH9-RD is typically of autosomal dominant inheritance, but more than 30% show *de novo* mutations [13].

Because of the risk of bleeding, renal biopsy was not done in most cases with nephritis caused by MYH9 gene mutations. In less than 20 patients in whom the biopsy data were reported main histological findings were mesangial expansion or proliferation in the earlier stage and focal segmental or global glomerulosclerosis (FSGS) in the late stage of renal disease. Electron microscopy (EM) showed thickening, splitting or attenuation of glomerular basement membrane (GBM) or focal podocyte foot process effacement [11, 14].

The pathogenesis of glomerulopathy in MYH9 disorders remains uncertain. NMMHC-IIA is expressed in podocytes, mesangial cells, in certain endothelial cells, and most tubular cells [15]. NMMHC-IIA is a major component of actin myosin contractile apparatus in the podocyte foot processes and has a role in maintaining and disassembling the slit diaphragm [16]. The mutated NMMHC-IIA, by altering the podocyte cyto-

skeleton, impairs the function and structure of the slit diaphragm resulting in proteinuria and the development of FSGS [11, 14]. It is unknown whether mesangial hypercellularity is due to direct effect of abnormal MYH9 protein on mesangial cells [16]. GBM abnormalities, often found in patients with MYH9-RD, raise the possibility that MYH9 mutations disrupt the ability of the podocyte to produce extracellular matrix proteins with the appropriate amount and stoichiometry or the ability to regulate the incorporation of these proteins into GBM in the process of physiologic remodeling [14].

Renin-angiotensin system blockade may be effective in reducing proteinuria of patients with progressive nephropathy caused by MYH9 mutations [14, 17]. The efficacy of ACE inhibitors in reducing proteinuria was observed in our patient, the long-standing effect could not be confirmed because of preterm therapy termination. Early recognition of MYH9-RD also offers a better quality of life to these patients [18].

Conclusion

Although MYH9-RD is a well-defined entity with precise diagnostic work-up recommendations available, the diagnosis is sometimes delayed due to misinterpretation of laboratory data, especially in the patients with *de novo* mutations and a negative family history. Reviewing peripheral blood smear to assess the platelet size should be mandatory. A high index of suspicion and understanding the pathophysiology of MYH9-RD is a clue to correct diagnosis. Screening for both nephritis and hearing impairment has to be considered in all patients with macrothrombocytopenia. Although there is no effective treatment for progressive renal disease and deafness, potentially harmful treatment of patients with thrombocytopenia caused by MYH9 mutations, such as intravenous immunoglobulin, corticosteroids or splenectomy could be avoided.

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