Research Article/ Araştırma Makalesi

Benign Thrombocytopenia in Childhood and Novel TURBB1, ANKRD26, and SAMD9 Variants

Çocukluk Çağında Benign Trombositopeni ve Yeni TURBB1, ANKRD26 ve SAMD9 Varyantları

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ABSTRACT

Aim: Thrombocytopenia is a common hematologic finding in children. This study evaluated the demographic, laboratory and genetic characteristics and prognosis of children with thrombocytopenia.

Material and Method: This retrospective study included children (n=82) examined with thrombocytopenia at Düzce University Faculty of Medicine Pediatric Hematology-Oncology Clinic between December 2021 and August 2023. Laboratory, clinical, and treatment characteristics of patients with idiopathic thrombocytopenic purpura (n=41) and without thrombocytopenia (n=41) were compared. Gene analysis was performed by clinical exome next-generation sequencing in selected cases.

Results: Children without idiopathic thrombocytopenic purpura (ITP) (n=41) had higher rates of fever (p<0.001), infection (p<0.001), cytopenia or pancytopenia (p=0.013) and pallor (p=0.014) than children with ITP (n=41). The median platelet count was significantly lower (p<0.001) and neutrophil levels (mean ± SD) (p=0.003) were higher in patients with ITP compared to patients without ITP. In children with infection (n=22), high fever (p<0.001), pallor (p<0.001), cytopenia and pancytopenia (p=0.04) were more frequent and mean platelet levels (± SD) and neutrophil levels (p=0. 004) were lower than those without infection (n=60). The median duration of thrombocytopenia (15 days vs. 90 days) (p=0. 04) was shorter in the infected group. Three novel variants were identified by clinical exome next-generation sequencing analysis in a boy with mild macrothrombocytopaenia. Three novel variants in one patient in the genes; a three :c. 340A >G (p. Arg114Gly) variant, a 002G>A (p. Asp668Asn) variant in the ANKRD26 gene in the SAMD9 gene and in the TUBB1 gene a 1342 G>T (p. Asp448Tyr) variant. The new TUBB1 variant was consistent with the patient's clinical presentation.

Conclusion: Infection-associated thrombocytopenia improves faster with higher platelet counts than ITP. Clinical exome next-generation sequencing analysis is recommended in cases with atypical ITP to diagnose congenital macrothrombocytopaenia.

Keywords: Idiopathic thrombocytopenic purpura, molecular genetics, thrombocytopenia, child.

ÖZ

Amaç: Trombositopeni çocuklarda sık görülen bir hematolojik bulgudur. Bu çalışmada trombositopenili çocukların demografik, laboratuvar, genetik özelliklerinin ve prognozlarının değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Bu retrospektif çalışmaya Aralık 2021-Ağustos 2023 tarihleri arasında Düzce Üniversitesi Tıp Fakültesi Çocuk Hematoloji-Onkoloji Polikliniği'nde trombositopeni tanısı konulan çocuklar (n=82) dahil edildi. İdiyopatik trombositopenik purpurası (n=41) olan ve olmayan (n=41) trombositopenili olguların laboratuvar, klinik, ve tedavileri karşılaştırıldı. Seçilmiş olgularda klinik ekzom yeni nesil sekanslama ile gen analizi yapıldı.

Bulgular: İmmun trombositopenik purpura (İTP)'li olmayan çocuklarda (n=41) İTP'lilere göre (n=41) ateş (p<0,001), enfeksiyon (p<0,001), bisitopeni veya pansitopeni (p=0,013) ve solukluk (p=0,014) oranları daha yüksekti. İTP'li hastalarda, İTP'li olmayan hastalara göre trombosit sayısının ortanca değeri (p<0,001) anlamlı olarak daha düşük ve ortalama nötrofil düzeyleri (±SD) (p=0,003) daha yüksekti. Enfeksiyonu olan çocuklarda (n=22), enfeksiyonu olmayanlara (n=60) göre yüksek ateş (p<0,001), solukluk (p<0,001), bisitopeni ve pansitopeni (p=0,04) daha sık ve ortalama trombosit düzeyleri (± SD) ile nötrofil düzeyleri (p=0,004) daha düşük bulundu. Ortanca trombositopeni süresi (15 güne karşın 90 gün) (p=0,04) enfekte grupta daha kısaydı. Hafif makrotrombositopenisi olan bir erkek çocukta klinik ekzom yeni nesil sekanslama analizinde üç yeni variant tanımlandı. ANKRD26 geninde 3:c. 340A >G (p. Arg114Gly) varyantı, SAMD9 geninde 002G>A (p. Asp668Asn) varyantı ve TUBB1 geninde 1342 G>T (p. Asp448Tyr) varyantı saptandı. Yeni TUBB1 varyantı hastanın kliniği ile uyumlu bulundu.

Sonuç: Enfeksiyona bağlı trombositopeni, İTP'ye göre daha yüksek trombosit sayıları ile daha hızlı iyileşir. Konjenital makrotrombositopeni tanısı için atipik İTP'li olgularda klinik ekzom yeni nesil sekanslama analizi önerilir.

Anahtar Kelimeler: İdiyopatik trombositopenik purpura, moleküler genetik, trombositopeni, çocu.

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Table 1: Demographics, clinics, and laboratories of children with thrombocytopenia.

		Min-Max			Median	Mean±SD/n-%		
Age (years)		0.00	-	17.0	7.0	8.0	±	5.0
Gender	Female					35		42.7%
	Male					47		57.3%
Fever (+)						11		13.4%
Pallor (+)						17		20.7%
Hematologic Diseases								
No						34		41.5%
Yes						48		58.5%
	ITP					41		50.0%
	Non-diagnosed					4		4.9%
	Aplastic anemia					2		2.4%
	MDS					1		1.2%
Malignancy (+)						0		0%
Infections (+)						22		26.8%
Drug-related (+)						2		2.4%
Pseudothrombocytopenia						3		3.7%
Laboratory at diagnosis								
Hemoglobin (g/dL)		7.0	-	20.6	12.0	12.3	±	2.0
Mean platelet volume (fL)		6.7	-	15.6	9.2	9.4	±	1.6
Platelet ($x10^3$)		2.0	-	148.0	64.0	63.7	±	51.2
Platelet ($x10^3$)								
	<20					29		35.4%
	20-50					8		9.8%
	>50	0.00	-	12400	3450	45		54.9%
Neutrophil ($/mm^3$)						3693	±	2416
Bicytopenia or pancytopenia						22		26.8%
Remission								
	(-)	1.00	-	1500	30.0	27		32.9%
	(+)					55		67.1%
Period of thrombocytopenia (day)						156	±	212
Bone marrow aspiration						44		53.7%
Intravenous immunoglobulin						30		36.6%
Thrombocyte transfusion						5		6.1%

Table 2. Comparison of the thrombocytopenic children with or without ITP.

		ITP (-)			ITP (+)			P- value	
		Mean±SD/n-%		Median	Mean±SD/n-%		Median		
Age		8.6 ± 5.3		9.0	7.4 ± 4.6		7.0	0.318	m
Gender	Female	15	36.6%		20	48.8%		0.264	X^2
	Male	26	63.4%		21	51.2%			
Fever	**(-)**	30	73.2%		41	100%		0.000	X^2
	(+)	11	26.8%		0	0.0%			
Pallor	**(-)**	28	68.3%		37	90.2%		0.014	X^2
	(+)	13	31.7%		4	9.8%			
Infection	**(-)**	22	53.7%		38	92.7%		0.000	X^2
	(+)	19	46.3%		3	7.3%			
Malignancy	**(-)**	41	100%		41	100%		1.000	X^2
	(+)	0	0.0%		0	0.0%			
Hemoglobin (g/dL)		12.4 ± 2.6		12.2	12.2 ± 1.3		12.0	0.888	m
Mean platelet volume (fL)		9.2 ± 1.3		9.0	9.6 ± 1.8		9.5	0.419	m
Platelet count (x10^3/mm^3)		98.5 ± 39.0		111.0	28.9 ± 36.1		11.0	0.000	m
Platelet count (x10^3/mm^3)	**<20**	3	7.3%		26	63.4%		0.000	X^2
	20-50	2	4.9%		6	14.6%			
	>50	36	87.8%		9	22.0%			
Neutrophil (x/mm^3)		2811 ± 1724		2760	4574 ± 2692		3840	0.003	m
Remission	**(-)**	17	41.5%		10	24.4%		0.100	X^2
	(+)	24	58.5%		31	75.6%			
Duration of thrombocytopenia		135 ± 160		30.0	177 ± 254		60.0	0.056	m
Bone marrow aspiration	**(-)**	30	73.2%		8	19.5%		0.000	X^2
	(+)	11	26.8%		33	80.5%			
Intravenous immunoglobulin	**(-)**	39	95.1%		13	31.7%		0.000	X^2
	(+)	2	4.9%		28	68.3%			
Thrombocyte transfusion	**(-)**	37	90.2%		40	97.6%		0.166	X^2
	(+)	4	9.8%		1	2.4%			
Bicytopenia/ Pancytopenia	**(-)**	25	61.0%		35	85.4%		0.013	X^2
	(+)	16	39.0%		6	14.6%			
Pseudothrombocytopenia	**(-)**	38	92.7%		41	100%		0.241	X^2
	(+)	3	7.3%		0	0.0%			
Drug-related	**(-)**	39	95.1%		41	100%		0.494	X^2
	(+)	2	4.9%		0	0.0%			

X^2 Chi-square test / m Mann-Whitney U test. ITP (idiopathic thrombocytopenic purpura), SD (standard deviation).

Table 3. Comparing the thrombocytopenic children with or without infection.

		Infection (-)			Infection (+)			P- value	
		Mean±SD/n-%		Median	Mean±SD/n-%		Median		
Age		8.2 ± 5.0		7.3	7.4 ± 5.0		6.0	0.486	m
Gender	Female	26	43.3%		9	40.9%		0.844	X^2
	Male	34	56.7%		13	59.1%			
Fever	**(-)**	59	98.3%		12	55%		0.000	X^2
	(+)	1	1.7%		10	45.5%			
Pallor	**(-)**	54	90.0%		11	50.0%		0.000	X^2
	(+)	6	10.0%		11	50.0%			
ITP	**(-)**	22	36.7%		19	86.4%		0.000	X^2
	(+)	38	63.3%		3	13.6%			
Hematological disease	**(-)**	16	26.7%		18	81.8%		0.000	X^2
	(+)	44	73.3%		4	18%			
ITP		38	63.3%		3	14%			
Non-diagnosed		4	6.7%		0	0.0%			
AA		1	1.7%		1	4.5%			
MDS		1	1.7%		0	0.0%			
Hemoglobin (g/dL)		12.3 ± 1.7		12.0	12.1 ± 2.9		11.9	0.402	m
Mean platelet volume (fL)		9.5 ± 1.7		9.4	9.2 ± 1.2		9.2	0.630	m
Platelet count (x10^3/mm^3)		53.4 ± 49.7		34.5	91.8 ± 45.2		103.0	0.005	m
Platelet count (x10^3/mm^3)	**<20**	26	43.3%		3	13.6%		0.034	X^2
	20-50	6	10.0%		2	9.1%			
	>50	28	46.7%		17	77.3%			
Neutrophil (x/mm^3)		4112 ± 2390		3690	2548 ± 2140		1550	0.004	m
Remission	**(-)**	21	35.0%		6	27.3%		0.509	X^2
	(+)	39	65.0%		16	72.7%			
Duration of thrombocytopenia		160 ± 152		90.0	143 ± 328		15.0	0.040	m
Bone marrow aspiration	**(-)**	22	36.7%		16	72.7%		0.004	X^2
	(+)	38	63.3%		6	27.3%			
Intravenous immunoglobulin	**(-)**	34	56.7%		18	81.8%		0.036	X^2
	(+)	26	43.3%		4	18.2%			
Thrombocyte transfusion	**(-)**	58	96.7%		19	86.4%		0.117	X^2
	(+)	2	3.3%		3	13.6%			
Bicytopenia/ Pancytopenia	**(-)**	49	81.7%		11	50.0%		0.004	X^2
	(+)	11	18.3%		11	50.0%			
Pseudothrombocytopenia	**(-)**	57	95.0%		22	100%		0.560	X^2
	(+)	3	5.0%		0	0.0%			
Drug-related	**(-)**	58	96.7%		22	100%		1.000	X^2
	(+)	2	3.3%		0	0.0%			

X^2 Chi-square test / m Mann-Whitney U test. ITP (idiopathic thrombocytopenic purpura), SD (standard deviation).

Table 4. Genetic mutations in a patient with mild thrombocytopenia.

Gene	Nucleotide-Protein Conversion	Zygosity	dbSNP	Effect	Effect Disease (Inheritance, OMIM#
ANKRD26	NM_ 014915.3:c.340A>G p.Arg114Gly	heterozygous	rs762754151	nonsynonymous_SNV	Trombocytopenia2;AD;188000
SAMD9	NM_ 001193307.1:c.2002G>A p.Asp668Asn	heterozygous	rs746976269	nonsynonymous_SNV	STERILE ALPHA MOTIF DOMAIN-CONTAINING PROTEIN 9; SAMD9610456
TUBB1	NM_ 030773.4:c.1342 G>T p.Asp448Tyr	heterozygous	-	nonsynonymous_SNV	Macrothrombocytopenia, otosomal dominant, TUBB1 related;AD;613112

dbSNP: Single Nucleotide Polymorphism Database, AD: autosomal dominant, SNV: single nucleotide variant.

Discussion

This retrospective study examined the profile of children with thrombocytopenia who were admitted to our pediatric hematology clinic. Thrombocytopenia due to hematological diseases (58.5%) was dominant. Among 48 hematological disorders, 41 patients had ITP.

Septicaemia was the most common diagnosis in a prospective study of 246 children with thrombocytopenia, followed by megaloblastic anemia, undiagnosed fever, local infection, hepatitis, and scrub typhus. They determined that fever, pallor, bleeding manifestations, lymphadenopathy, and splenomegaly were present. Bleeding, arthralgia, rash, pallor, GI symptoms, hematological disorders, and malignancy were associated with severe thrombocytopenia (2).

Our study revealed that having ITP was strongly associated with lower platelet counts, fever, infection, pancytopenia, and pallor rates. Bone marrow aspiration procedure and IVIG treatment were more commonly applied to patients with ITP. Unexpectedly, MPV levels at diagnosis were not statistically higher in the ITP than in the non-ITP group; remission rates and duration of thrombocytopenia were also similar.

In another study comparing primary ITP with secondary ITP and non-ITP, the leading diagnoses were equally infectious and autoimmune disorders. They also mentioned that genetic disorders for thrombocytopenia were misdiagnosed for ITP due to unavailable genetic studies. Non-ITP thrombocytopenias demonstrated higher platelet counts than infection or autoimmune-associated secondary ITP. Secondary ITP and non-ITP patients did not require therapy in this study, so they concluded that people with severe bleeding need expanded evaluation (3). In a retrospective analysis of pediatric thrombocytopenia, the authors reported that the cumulative incidence of remission was significantly higher in post-immunization and post-viral infection (compared with primary ITP patients) but worse in autoimmune diseases and immunodeficiencies patients (4). Intravenous immunoglobulin (IVIG) is the mainstay treatment of ITP and was more efficient alone or combined with steroids than steroids alone (5).

Our study showed that infection-related thrombocytopenia was associated with fever, pallor, pancytopenia, and lower neutrophil and thrombocyte levels. Our patients with thrombocytopenia and infection recovered more rapidly than other causes. Due to higher platelet counts and rapid recovery, bone marrow aspiration and biopsies were rarely performed in the infection group. IVIG is one of the main treatments for ITP, so IVIG treatment rates are higher in the non-infection group. These results support our finding that most children with infections did not have ITP (86.4%).

MPV (mean platelet volume) was increased in ITP and was significantly higher in chronic ITP than in acute and persistent ITP, with a cutoff value of 8.7 fL. Thus, thrombocyte size is critical for diagnosing inherited thrombocytopenias (6). Macrothrombocytopaenia (like Gray Platelet Syndrome) also have increased MPV (7). TUBB1 gene variants are associated with autosomal dominant isolated macrothrombocytopenia-1 (MACTHC1), large platelets with irregular numbers and irregular shapes. Various TURBB1 variants associated with macrothrombocytopaenia were reported in studies. Affected individuals in this disease are reported not to have increased bleeding episodes, and platelet function is normal; macrothrombocytopaenia is usually an incidental laboratory finding (8-12). Our study revealed a novel variant of (c.1342G>T) (p.Asp448Tyr) on the TUBB1 gene. ACMG rules defined this mutation as a VUS (change of uncertain significance) relative to PM2. Our index case was consistent with this mutation due to macrothrombocytopaenia and no bleeding.

Variants in the ANKRD26 gene are associated with Thrombocytopenia-2 (THC2), an autosomal dominant, non-syndromic disorder characterized by a reduced average platelet count resulting in a mild bleeding tendency. Laboratory studies show no defects in platelet function or morphology, and bone marrow examination shows standard numbers of megakaryocytes and normal maturation stages, suggesting defective platelet production or release (13). Our patient with the TUBB1 novel variant also had an unknown change with VUS in the ANKRD26 gene (c.340A>G)(p.Arg114Gly) found in one allele (heterozygous). However, our patients' clinic was inconsistent with that mutation due to no significant bleeding history.

SAMD9 gene variants are related to Monosomy 7 myelodysplasia and leukaemia syndrome 2 (14). Our study revealed a VUS change (c.2002G>A)(p.Asp668Asn) in this gene with a DANN score of 0.992. Our index case does not fill the MDS criteria. However, close follow-up is required for the possible MDS development. All three mutations defined in the same patients must be verified by gene-gene sanger sequencing and familial segregation.

Conclusion

This retrospective study revealed that having ITP was strongly associated with lower platelet counts, fever, infection, pancytopenia, and pallor rates. Bone marrow aspiration procedure and IVIG treatment were more commonly applied to patients with ITP. Unexpectedly, MPV levels at diagnosis were not statistically higher in the ITP than in the non-ITP group, and remission rates and duration of thrombocytopenia were also similar. Our study demonstrated that infection-related thrombocytopenia was associated with fever, pallor, pancytopenia, and lower neutrophil and higher thrombocyte levels. Our patients with thrombocytopenia and infection recovered more rapidly than other causes. Due to higher platelet counts and rapid

recovery, bone marrow aspiration and biopsies were found to be rarely performed in the infection group. The index case with macrothrombocytopaenia had three novel mutations: one was VUS (c.2002G>A)(p.Asp668Asn) in the SAMD9 gene, one was VUS in the ANKRD26 gene (c.340A>G)(p.Arg114Gly), and the other was (c.1342G>T)(p.Asp448Tyr) variant on the TUBB1 gene which revealed clinical relevance with the patient's clinic. After performing gene-gene sanger sequencing and familial segregation, the verification of this disease will be possible.

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