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Full Length Article

The influence of specific mutations in the AT gene (*SERPINC1*) on the type of pregnancy related complications

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ABSTRACT

Background: Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder, caused by mutations in the *SERPINC1* gene. The most common clinical presentation in AT deficient patients includes venous thrombosis and pulmonary embolism, while the association of AT deficiency and its effect on the development of pregnancy complications has been less studied. The aim of our research was to evaluate the effect of AT deficiency types, determined by genotyping, on pregnancy outcomes.

Methods: A retrospective cohort study included 28 women with AT deficiency, and their 64 pregnancies were analyzed.

Results: With regard to live birth rate, a significant difference was observed among women who were carriers of different *SERPINC1* mutations, as the rate varied from 100% in cases of type I to the extremely low rate of 8% for women with type II HBS (AT Budapest 3) in the homozygous variant, P = 0.0005. All pregnancies from the type I group, even untreated ones, resulted in live births. In women with AT Budapest 3 in homozygous variant the overall live birth rate increased to 28.5% in the treated pregnancies. In this group the highest incidence of fetal death was observed of 62%; repeated fetal losses in 30%; fetal growth restriction in 22% and placental abruption in 7% of all pregnancies.

Conclusion: Our study results indicate a difference between type I and type II AT deficiency. The risk of pregnancy related VTE was equally present in both groups, except for AT Budapest 3 in the heterozygous variant, while adverse pregnancy outcomes were strictly related to type II, especially AT Budapest 3 in the homozygous variant.

1. Introduction

Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder, caused by mutations in the AT gene (*SERPINC1*) [1,2]. The gene for human AT (*SERPINC1*) is located at position 1q23–q25 and > 330 different mutations have been reported so far (http://www.hgmd.cf.ac.uk; last accessed at July 30, 2018) [3]. AT deficiency is classified into two types. Type I is a quantitative disorder characterized by both decreased amount and activity of AT. Type II is a functional disorder classified into three subtypes according to the site of causative mutation. Thus, type II reactive site (RS) is caused by a defect in the AT RS; type II heparin-binding site (HBS) due to a mutated HBS and type II pleiotropic (PE) caused by pleiotropic mutations [1,4,5]. The prevalence of inherited AT deficiency in the general population is

Abbreviations: SERPINC1, serine protease inhibitors; AT, antithrombin; HBS, heparin-binding site; RS, reactive site; PE, pleiotropic; DVT, deep venous thrombosis; PE, pulmonary embolism; VTE, venous thromboembolism; FGR, fetal growth restriction; RFL, recurrent fetal loss; IUFD, intrauterine fetal death after the 20th week of gestation; MLPA, multiplex ligation-dependent probe amplification; LMWH, low molecular weight heparin; APC-R, activated protein C resistance; LA, lupus anticoagulant

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Table 1
Characteristics of the study population.

	Type I Heterozygous	Type II HBS Homozygous (AT Budapest 3)	Type II HBS Heterozygous (AT Budapest 3)	Type II PE (Pleiotropic) Heterozygous
Number of participants	11	5	10	2
Current age, mean (range)	44.8 (23–64)	33.5 (29–35)	51.3 (29–89)	50.5 (40–61)
Age at diagnosis, mean (range)	36 (19–60)	25.6 (19–34)	46.9 (22–87)	49.5 (39–60)
AT activity [IU/ml]				
Berichrom ATIII (anti-IIa assay)	0.48 (0.33–0.62)	0.57 (0.36–0.79)	0.76 (0.59–0.92)	0.53 (0.52–0.52)
Innovance AT (anti-Xa)	0.55 (0.47–0.68)	0.19 (0.13–0.27)	0.53 (0.42–0.62)	/
STA-StachromAT III (anti II assay)	0.51 (0.49–0.72)	0.25 (0.23–0.29)	0. 58 (0.52–0.70)	/
LIATEST ATIII [%] mean (range)	56 (49–75)	125 (93–143)	94 (93–119)	/
SERPINC1 mutations; family (number of pts)	c.652-654 del, p.Ile218 del *heterozygous*; 1 (2), 2 (1) c.1019-1022 del, p.Leu340LeufsTer5 *heterozygous*; 3 (2) c.1171 C > T, p.Arg391[a] c.1219(-1)G > C *heterozygous*; 4 (1) c.415-416Aadel, p.Lys139(171)Valfsx16 *heterozygous*; 5 (2) c.548del,p.Ser183SetfsTer100 *heterozygous*; 6 (1) IVS5 + 27[a] G > C *heterozygous*, *IVS1-18C > G heterozygous*; 7 (2)	c.391 C > T, p.Leu99(131)Phe (AT Budapest 3) *homozygous*; 1–5 (1)	c.391 C > T, p.Leu99(131)Phe (AT Budapest 3) *heterozygous*; 5 (1), 6 (1), 7 (1), 8 (2), 9 (2), 10 (1), 11 (2)	c.1301T > C, p.Phe402(434)Ser (AT Torino) *heterozygous*; 1 (2)
FVLeiden/*FII G*20210A	1/0	/	2/0	/

AT activity was expressed as mean (range).
Differences between tests for AT activity in type II HBS (Ho): Berichrom vs Innovance, P = 0.005; Berichrom vs Stachrom, P = 0.008
Innovance vs Stachrom, P = 0.333
Differences between tests for AT activity in type II HBS (Hz): Berichrom vs Innovance, P = 0.006; Berichrom vs Stachrom, P = 0.03;
Innovance vs Stachrom, P = 0.553.

[a] Two members were carriers of two silent mutations in heterozygous variant.

Table 2
Clinical presentation among women with AT deficiency.

	Type I Heterozygous	Type II HBS Homozygous (AT Budapest 3)	Type II HBS Heterozygous (AT Budapest 3)	Type II PE (Pleiotropic) Heterozygous
Number of participants	11	5	10	2
Number of pts. with thrombosis n (%)	9 (82)	4 (80)	2 (20)	2 (100)
Age at the first thrombosis, mean (range)	25.7 (19–35)	24 (17–33)	59.5 (35–84)	30 (20–40)
Localization of the first VTE				
Proximal DVT	5	3	0	2
Distal DVT	1	0	1	0
DVT/PE	1	0	1	0
PE	1	1	0	0
Thrombosis sinus cerebri	1[a]	0	0	0
Number of patients with recurrent thrombosis n (%)	4 (44)	3 (60)	1 (10)	1
Age at the time of recurrent thrombosis, mean (range)	41.1 (22–60)	24.0 (19–28)	36 (/)	42 (/)
Total number of VTE	22	8	3	3
Total number of stroke/MI	0	1	0	1

VTE-venous thromboembolism; DVT-deep venous thrombosis; PE-pulmonary embolism, MI-myocardial infarction.
Differences between type I and type II with regard to: Number of pts with thrombosis - type I/type II HBS (Ho), P = 1.0 (OR = 1.125; 95% CI 0.07–16.30); type I/type II HBS (Hz), P = 0.008 (OR = 18.00; 95% CI = 2.03–159.09); type I/type II PE, P = 0.516 (OR = 4.50; 95% CI = 0.37–54.15). Age at the first and recurrent thrombosis type I/type II HBS (Homozygous), P = 0.138; P = 0.176.
[a] PE as a complication (developed 10 days after hospital admission).

Table 3
Overall pregnancy outcome in relation to the type of AT deficiency.

	Type I Heterozygous	Type II HBS Homozygous (AT Budapest 3)	Type II HBS Heterozygous (AT Budapest 3)	Type II PE (Pleiotropic) Heterozygous
Number of participants	11	5	10	2
Total number of pregnancies	12	27	17	8
Live birth rate (%)	12 (100)	2[a] (8)	16 (94)	4 (50)
Pregnancy complications, n (%)				
RFL	0	8 (30)	0	4 (50)
IUFD	0	17 (62)	0	0
Placental ab.	0	2[b] (7)	1[b] (6)	0
FGR	0	6 (22)	0	0
VTE	4 (33)	4 (15)	0	2 (25)
Stroke	0	1 (4)	0	0
Hormone related VTE	2	0	0	0

HBS - heparin binding site; RFL - recurrent fetal losses, IUFD - intrauterine fetal death after 20th gestation week; FGR - fetal growth restriction; VTE - venous thromboembolism.
Differences between type I and type II with regard to the live birth rate: type I/type II HBS (Ho), P = 0.0005 (OR = 255.00; 95% CI = 11.36–5722.62); type I/type II HBS (Hz), P = 0.6241 (OR = 2.27; 95% CI = 0.08–60.63); type I/type II PE, P = 0.0428 (OR = 25.00; 95% CI = 1.11–562.85).
[a] Preterm delivery at gestation week 29 and 33 due to intrauterine growth restriction.
[b] Placental abruption resulting in fetal loss.

estimated to be between 1: 2000 and 1:3000 [6], while the prevalence of AT deficiency in patients with venous thromboembolism (VTE) is much higher, between 1:20 and 1:200 [7]. Based on our previously reported studies AT deficiency with an incidence of 5.8% among selected Serbian patients with VTE was obtained, while in women with pregnancy related VTE total incidence of all inhibitors, with predominantly presence of AT deficiency was 6% [8,9]. The primary role of AT in the coagulation system is inhibition of thrombin and factor Xa, but it also inhibits factors IXa, XIa and XII [1,10,11].

Clinical manifestation and severity of the disease vary depending on the type of AT deficiency and the site of mutation. The most common clinical presentation in AT deficient patients includes deep venous thrombosis (DVT) and pulmonary embolism (PE), and its influence on the occurrence of thrombosis has been shown in numerous studies [7,12–18]. However, effects on the development of pregnancy complications have been less studied. Results from a large meta-analysis showed strong association of AT deficiency with pregnancy related VTE and late pregnancy loss. However, for early pregnancy loss and placental abruption associations were weak, while no data for analysis were given for the development of fetal growth restriction (FGR) [19]. The studies included in the mentioned meta-analysis had no information on the genetic background of AT deficiency. On the other hand, recently published results involving seven women with AT deficiency during 18 pregnancies, showed that AT deficiency was an important risk for the development of all forms of pregnancy related complications [20]. It should be emphasized that very few data have been related to specific mutations in the *SERPINC1* gene and their association with pregnancy related complications, such as recurrent fetal loss before the 20th gestation week (RFL), intrauterine fetal death after the 20th week of gestation (IUFD), FGR, placental abruption and preeclampsia. So far, the few studies in which the genetic background of AT deficiency has been presented, gave a brief overview [21] or case presentation of the effect of type II HBS on adverse pregnancy outcomes [22–25].

The aim of our study was to evaluate the effect of different types of AT deficiency, as determined by genotyping, on pregnancy outcomes in order to point to any differences with regard to the specific mutations.

2. Patients and methods

In order to evaluate the influence of specific genetic mutations in the *SERPINC1* gene, that cause different types of AT deficiency, on the type of pregnancy complications, we conducted a retrospective cohort study. The data from the Serbian AT deficiency Register were used. So

Table 4
Treated pregnancies with outcome in relation to the type of AT deficiency.

	Type I Heterozygous	Type II-HBS Homozygous (AT Budapest 3)	Type II-PE (Pleiotropic) Heterozygous
Women treated with anticoagulant therapy during pregnancy	1	5	1
Number of treated pregnancies	1	7[a]	1
Treatment (LMWH; AT concentrate)	Prophylactic LMWH dose, Nadroparin 3800 IU 1 ×/day from 20th GW; AT substitution before delivery and in postpartum period for three days		Therapeutic LMWH dose, Enoxaparin, 80 mg 2 ×/day, from 14th GW due acute DVT; AT substitution before delivery and in postpartum period for 5 days
Outcomes of pregnancies			
Delivery at term	1 at 38th GW	0	1 at 40th GW
Preterm delivery	0	2[b] (28.5)	0
RFL	0	2 (28.5)	0
IUFD	0	3[b] (43)	0
Pregnancy complication			
Placental ab.	0	1 (14)	0
FGR	0	3 (43)	0
VTE	0	3 (43)	0
Stroke	0	1 (14)	0

HBS - heparin binding site; RFL - recurrent fetal losses, IUFD - intrauterine fetal death after 20th gestation week; FGR - fetal growth restriction; VTE - venous thromboembolism; LMWH - low molecular weight heparin.

[a] Treatment of women with type II HBS (AT Budapest 3) in homozygous variant is shown in Table 5.

[b] In 2 participants VTE was recorded after preterm delivery or IUFD.

far, 77 subjects from 24 Serbian families were genotyped and included in the Register. Among them, 59 are carriers of specific mutation in *SERPINC1*. For the current study, nearly all women diagnosed with AT deficiency in three Serbian Thrombosis Centers from 1994 to 2017, who had had at least one pregnancy, were eligible. Four women, carriers of AT deficiency, didn't meet the criteria and they were not included in the study since they haven't been pregnant so far. Two of them with type II HBS in the homozygous variant had juvenile thrombosis and two with type I in the heterozygous variant are still asymptomatic.

AT deficiency was confirmed by family studies and genotyping. Originally, patients were referred for testing due to clinical manifestations of VTE or pregnancy related complications. 28 women from 19 families, aged over 18 were included in this study. They were divided into four groups as follows: type I deficiency [11], type II HBS (AT Budapest 3) [15] among whom five were homozygous and 10 had the heterozygous variant, while two subjects had type II PE in the heterozygous variant. The current mean age of the study participants in the group with type I deficiency was 44.8 years; for type II HBS in the homozygous variant 33.5 years, for type II HBS in the heterozygous variant 51.3 years and 49.5 years for those with type II PE.

All participants were approached with questionnaires about the number of pregnancies and the history of previous pregnancy complications, such as recurrent fetal loss (before and after gestation week 20), placental abruption, FGR, preeclampsia, as well as any therapy given during the pregnancy (anticoagulants and AT substitution), time and mode of delivery, pregnancy related thrombosis or hormone related thrombosis. Data about venous thrombosis or other thrombotic manifestations out of pregnancy, as well as records of any comorbidities were also collected. All data with regard to the laboratory and genetic results that confirmed AT deficiency status were taken from the Registry.

Institutional approval for the study was granted by the Local Research Ethics Committee (EK-number 2471/1) in accordance with internationally accepted ethical standards and each participant signed the informed consent form.

2.1. Laboratory methods

2.1.1. AT activity

In order to confirm the initial diagnosis of AT deficiency revealed by the thrombin based Berichrom Antithrombin III assay (Siemens Healthcare Diagnostics, Marburg, Germany), AT activity was determined in a new sample using a chromogenic assay based on FXa inhibition in the presence of heparin (Innovance Antithrombin; Siemens Healthcare Diagnostics, Marburg, Germany). Analyses were performed on a BCS XP coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany), applying the reference range designated as normal by the manufacturer: 79–111% and 83–119%, respectively.

In addition, the samples were analyzed using a second thrombin based assay (STA-StachromAT III, Diagnostica Stago, SAS, Asnieres sur Seine France) on STA Compact coagulation analyzer, applying the reference range designated as normal by the manufacturer (80–120%).

2.1.2. AT antigen

The antigen level was determined using the Microlatex Particle-Mediated Immunoassay (LIATEST ATIII, Diagnostica Stago, France), applying the reference range designated as normal by the manufacturer (80–120%).

2.1.3. Thrombophilia testing

All participants were tested for the presence of thrombophilia. This included assessment of protein C and protein S activity, activated protein C resistance (APC-R) and the presence of lupus anticoagulant (LA). For the detection of thrombophilia, Siemens tests were used and analyses were performed on a BCS XP coagulation analyzer (Siemens Healthcare Diagnostics, Marburg, Germany).

2.2. Genetic analysis

FV Leiden and FII G20210A mutations were genotyped as previously described [26,27].

The AT gene (*SERPINC1*) was analyzed by the gold standard Sanger fluorescent sequencing method in which all exons, exon-intron boundaries and the promoter region were examined. Fluorescent direct sequencing was carried out in an ABI PRISM 3130-Avant Genetic Analyzer (Applied Biosystems Foster City, CA) and Sequencing Analysis 5.4 software was used for the evaluation. In cases when no causative mutations were found by Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA) was performed according to the manufacturer's instructions using a SALSA MLPA KIT P227 (MRC-Holland, Amsterdam, the Netherlands). The MLPA products were analyzed by Gene Mapper Software 4.1 (Thermo Fisher Scientific).

Table 5
Summary of therapy, pregnancy outcome/complications in women with type II HBS (AT Budapest 3) in the homozygous variant.

	Year/age	Outcome	Birth	GW	Treatment during pregnancy	Peripartal treatment	Complication	Date of diag.
Case 1								
1	2000/18	Neg.	Miscarriage	16	None	None		
2	2001/19	Neg.	Miscarriage	16	None	None		
3	2002/20	Neg.	IUFD	20	None	None		
4	2002/21	Neg.	Miscarriage	20	None	None		
5	2003/22	Neg.	IUFD	21	None	None		
6	2004/23	Neg.	IUFD	24	None	None		
7	2005/24	Neg.	IUFD	22	None	None		
8	2006/25	Neg.	IUFD	24	None	None		
9	2007/26	Preterm delivery	C-section	33	From 6th GW Nadroparin 5700 sc. 1 ×/day	1 day before C-section and 3 days after, 2000 IU AT/day + Nadroparin 5700 IU after C-section for 6 weeks	FGR from 31st GW	2007
Case 2								
1	2003/29	Neg.	Miscarriage	16	None			
2	2004/30	Neg.	Miscarriage	16	None			
3	2007/34	Neg.	IUFD	23	From 16th GW Nadroparin 5700 sc. 1 ×/day	A day before C-section 2000 IU AT Nadroparin 5700 IU 1 ×/per day, after C-section for 4 weeks	FGR from 27 GW	2007
4	2008/35	Preterm delivery	C-section	29	From 6th GW Nadroparin 5700 IU sc. 2 ×/day	1 day before C-section and 3 days after, 2000 IU AT/day + Nadroparin 5700 2 ×/day after C-section for 6 weeks	Fatal PE 6 weeks after delivery	
Case 3								
1	2004/18	Neg.	IUFD	21	None			
2	2005/19	Neg.	IUFD	20	None			
3	2006/20	Neg.	IUFD	22	None			
4	200,721	Neg.	IUFD	27	None			2007[a]
5	2008/22	Neg.	IUFD	24	None		Placental ab. + DVT post C-se	
6	2009/23	Neg.	IUFD	22	None		Placental ab.	
7	2011/25	Neg.	IUFD	20	None			
8	2012/26	Neg.	FGR	20	From 6th GW Nadroparin 5700 sc. 1 ×/day From 12th GW Nadroparin 5700 IU + 3800 IU 2 ×/day + AT 2000 on the second day + Aspirin 100 mg	1 day before C-section and 3 days after, 2000 IU AT/day + Nadroparin 5700 IU 2 ×/day after C-section for 6 weeks	Stroke at 12th GW under LMWH FGR from 15th GW	2012
Case 4								
1	2000/18	Neg.	IUFD	24	None	None		
2	2006/24	Neg.	Miscarriage	8	UFH from 7thGW 600 IU/kg/day She was switched to therapeutic LMWH (200 IU Nadroparin/kg twice daily)		At 7th GW left iliac vein thrombosis, propagation of the thrombotic process into the opposite iliac vein and inferior vena cava	2006
3	2010/28	Neg.	Miscarriage	8	From 6th GW VKA was switched to Nadroparin 5700 IU + 2850 IU sc. 2 ×/day + AT 2000 IU on the second day	Long term VKA		
Case 5								
1	2006/28	Neg.	Miscarriage	8	None			1997
2	2007/29	Neg.	Miscarriage	10	None			
3	2008/30	Neg.	IUFD	22	From 6th GW she was switched from VKA to Nadroparin 5700 IU + 2850 IU sc. 2 ×/day From 8th GW AT 2000 IU on the third day	After delivery Nadroparin 5700 IU + 2850 IU sc. 2 ×/day	IUFD at 22nd GW, 8 days after delivery thrombosis v. portae, thrombosis v. jugularis, subcl, brachialis l sin	

The presented cases are indicated as family 1–5 in Table 1, GW - week of gestation, FGR - fetal growth restriction, IUFD - intrauterine fetal death after 20th gestation week, VKA – vitamin K antagonist.
[a] The first thrombophilia testing in 2007 showed normal AT activity.

2.3. Statistical methods

The Statistical Package for Social Sciences 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Student's *t*-test and Fisher's exact test were used to evaluate differences in the clinical characteristics of the study participants. Odds ratios (OR), 95% confidence intervals (CI) was estimated. The probability $P < 0.05$ was considered as statistically significant.

3. Results

Concerning their current age and their age at the time of diagnosis, women with type II HBS (AT Budapest 3) in the homozygous variant were younger as compared to women with other mutations. The values obtained for AT activity determined with three different tests were disparate. Namely, the originally recorded results for AT activity obtained using Berichrom AT, showed significantly higher levels of AT activity for type II HBS (AT Budapest 3) in comparison to the other tests. In a few cases, one from the group with type II HBS in the homozygous variant (Case 3, previously described in a case report [23]) and in three from the group with type II HBS in the heterozygous variant, the obtained results were even within the normal range. In their case, the diagnosis of AT deficiency was based on genetic analyses that were performed as part of a family study. The genetic results showed the presence of six different types of *SERPINC1* mutations that caused AT deficiency type I among 11 women from 7 families. In the family 7 the presence of two silent mutations were recorded in two subjects; mother who had pregnancy related DVT complicated with PE at age of 31, and daughter who had hormone related DVT at age of 19. Type II AT deficiency was confirmed in 17 subjects. In 15 women from 11 families, type II HBS with the already known mutation *c.391 C > T, p.Leu99(131) Phe* (AT Budapest 3) in homozygous or heterozygous variants was pointed. While, two women which originate from the same family, were carriers of the *c.1301T > C, p.Phe402(434)Ser* (AT Torino) in the heterozygous variant, which caused type II PE. Analyses of prothrombotic mutations showed that one woman with type I and two women with type II HBS were carriers of the FV Leiden mutation in the heterozygous variant (Table 1).

Analysis of the clinical characteristics in relation to the type of AT deficiency showed that thrombosis was equally prevalent in all groups, except for type II HBS (AT Budapest 3) in the heterozygous variant, where thrombotic events occurred in 20% of cases. In relation to the age of the first or recurrent thrombosis event, there was no difference between investigated groups, $P = 0.138$, $P = 0.176$. Arterial thrombosis, i.e. stroke or myocardial infarction were observed only in cases of type II HBS in the homozygous variant and for type II PE (Table 2).

In total, 64 pregnancies and outcomes of pregnancies were analyzed as shown in Table 3. With regard to the live birth rate, significant differences were observed among women carriers of different *SERPINC1* mutations, from 100% in type I to the extremely low rate of 8% for women with type II HBS (AT Budapest 3) in the homozygous variant, $P = 0.0005$. Homozygous type II HBS was predominantly associated with adverse pregnancy outcomes; with the highest incidence of IUFD observed in 62% of pregnancies; RFL in 30%; FGR in 22% and placental abruption in 7% of all pregnancies. The highest incidence of RFL (50%) occurred among the women who were carriers of type II PE. Pregnancy related VTE at the highest incidence of 33% was observed for type I deficiency, 25% in type II PE, 15% in type II HBS (AT Budapest 3) in the homozygous variant, while it was absent in cases of type II HBS (AT Budapest 3) in the heterozygous variant (Table 3).

Anticoagulant treatment was used during nine pregnancies in seven women. For women with type I and type II PE this resulted in positive outcomes; delivery at 38 or 40 weeks gestation. However, for women carriers of HBS (AT Budapest 3) in the homozygous variant, despite treatment, only 28.5% of pregnancies resulted in a live birth, all as preterm deliveries due to FGR. Likewise, 28.5% of pregnancies ended as RFL, while 43% resulted in IUFD.

In four women thrombosis appeared during the course of anticoagulant therapy (Case 4 and Case 5, previously described in a case report [22]). Two of them developed deep venous thrombosis (DVT), one in early pregnancy, the other 8 days after delivery. One woman developed massive PE with a fatal outcome 6 weeks after delivery, while another suffered a stroke in early pregnancy (Case 3, previously described in a case report [23]). The therapy offered to AT deficient women during pregnancy varied as well as the indication for introduction of anticoagulant therapy. A woman carrier of type II PE AT deficiency was given therapeutic doses of LMWH (80 mg of Enoxaparin sodium every 12 h) from the 14th gestation week due to acute DVT during the second pregnancy. Pregnant woman from the group with type I deficiency, even asymptomatic, received prophylactic doses of low molecular weight heparin (LMWH), 3800 IU of Nadroparin once daily, from the 20th gestation week during her first pregnancy. Anticoagulant treatment in her case, was introduced based on the individual risk assessment, considering the fact that her mother developed DVT in both pregnancies (Table 4).

Pregnant women carriers of HBS (AT Budapest 3) in the homozygous variant, received a high prophylactic LMWH doses from early pregnancy. In three pregnancies, Nadroparin was prescribed twice daily due to previous DVT/RFL, while in four cases Nadroparin was given once daily due to RFL. All of them were prepared for delivery or Cesarean section using AT concentrates one day before delivery or surgery. The use of AT substitution was continuous during the following two or three days after delivery. AT concentrates were given over longer periods during two pregnancies in two women who had been substituted from the 12th and the 16th gestation week of pregnancy respectively.

Among the 27 pregnancies observed among women with HBS (AT Budapest 3) in the homozygous variant, 20 were untreated. In 18 cases this was because AT deficiency had not been recognized at the time of pregnancy, while in one case the first two pregnancies ended very early, before therapy was introduced. It should be pointed out that in Case 3 the first thrombophilia testing did not reveal AT deficiency, and three subsequent pregnancies remained untreated. In this particular case, the diagnosis of AT deficiency was missed because an insufficiently sensitive test for revealing type II HBS deficiency was used (Table 5).

4. Discussion

Data on the association between specific genetic mutations at the level of the *SERPINC1* gene and pregnancy outcomes or pregnancy complications, have been presented so far mainly as case reports and mostly related to type II HBS in the homozygous variant [22–25]. In this study, we evaluated pregnancy outcomes and pregnancy complications among AT deficiency women with different types of mutations. To the best of our knowledge, our cohort on pregnancy outcomes is still the largest ever published.

Our results showed that the risk of pregnancy related thrombosis was equally present in both groups, while adverse pregnancy outcomes were strictly related to type II HBS (AT Budapest 3) in the homozygous variant. Namely, we have shown that AT deficiency is a strong risk factor for pregnancy related VTE regardless of type, except for type II HBS (AT Budapest 3) in the heterozygous variant. In untreated pregnancies the highest incidence of VTE was observed in women carriers of type I deficiency. In the treated pregnancies, type II HBS (AT Budapest 3) in homozygous variant was associated with a high risk of VTE and stroke.

All pregnancies from the type I group, even untreated, resulted in live births, without obstetric complications, except for VTE, which appeared as a complication during pregnancy in 33% of cases. In the group with type II HBS (AT Budapest 3) in the homozygous variant, all untreated pregnancies had an unfavorable outcome. The overall live birth rate in this group was raised to 28.5% for treated pregnancies,

although all types of pregnancy complications occurred, despite anticoagulant therapy. Late fetal loss was the most common, as almost half of the pregnancies (43%) resulted in IUFD. The occurrence of FGR preceded all cases of late pregnancy loss. Likewise, signs of FGR were the main reason for preterm delivery in the pregnancies that resulted in live births. Unfortunately, early pregnancy loss was also frequent with an incidence of 28.5%. On the other hand, for women with type II HBS (AT Budapest 3) in the heterozygous variant even untreated pregnancies had a high live birth rate of 94%. Untreated pregnancies in women with type II PE resulted in a live birth rate of 50%. Their pregnancies ended with no obstetrical complications, except for the development of pregnancy related DVT in both women, in one during the first and in the other during the second pregnancy.

Successful pregnancy outcomes in patients with thrombophilia depend on their management. Considering that AT deficiency is a rare and highly thrombogenic condition, management of pregnant women with this thrombophilic disorder is challenging [28,29]. However, well designed clinical studies that would result in strong recommendations regarding the appropriate dose of LMWH and the use of substitution therapy in pregnant women with AT deficiency are still lacking [7]. So far, management strategies in pregnant women with AT deficiency have largely been based on case reports and expert opinion [30]. Alguel et al. reported successful pregnancies in a woman with type II HBS (AT Budapest 3) in homozygous variant without AT replacement during pregnancy, in the first pregnancy with LMWH, and in the second one with vitamin K-antagonists. They concluded that women with homozygous AT type II HBS deficiency may have successful outcomes without AT replacement during pregnancy [31]. Bearing in mind our results, the same conclusions can be drawn only for women with type I deficiency. However, analyses of pregnancy outcomes in the group with type II HBS (AT Budapest 3) in homozygous variant, indicate that adverse pregnancy outcomes and all pregnancy complications were represented regardless of the applied therapy. Some women with this type of AT deficiency have a substantial risk of thrombosis during the peripartal period despite appropriate medical treatment (high prophylactic doses of LMWH and the use of AT concentrate). Moreover, complications in prior pregnancies in combination with the previous thrombosis may minimise the chance of a positive outcome of pregnancy. Similarly to our results, earlier data pointed to the severe clinical features and frequent adverse pregnancy outcomes, especially in untreated pregnancies [20,24,25]. They agree that appropriate treatment is the only way to reduce the incidence of pregnancy complications among women with AT deficiency. It should be noted that timely diagnosis of AT deficiency as well as effective treatment based on individual risk assessments, represent the first steps in the improvement of pregnancy outcome. Unrecognized type II HBS AT deficiency, due to already known difficulties in detecting this disorder with standard functional assays [32], may have an impact on deterioration of the clinical picture. The difficulties in detecting AT activity have been proven in our study too, that is especially well documented through the Case 3. Namely, the first thrombophilia testing in 2007 showed normal AT activity. Therefore, her three subsequent pregnancies remained untreated, that resulted in adverse pregnancy outcomes.

Our study has a limitation that should be considered. Namely, the number of patients is relatively small, especially in relation to type II PE. Likewise, our study didn't include the carriers of other mutations causing type II HBS deficiency. Additionally, our study is a retrospective cohort study, where the long time between the occurrences of the thrombotic events and of the pregnancies could have an implication that resulted in recall bias. However, since this severe inherited thrombophilia is a very rare disorder, our cohort on pregnancy outcomes is still the largest ever published.

5. Conclusion

Our study results indicate a difference between type I and type II AT deficiency. The risk of pregnancy related VTE was equally present in both groups except in the case of type II HBS (AT Budapest 3) in the heterozygous variant. While, adverse pregnancy outcomes were strictly related to type II, especially HBS (AT Budapest 3) in the homozygous variant. Close monitoring with appropriate anticoagulant treatment alone or in combination with AT substitution, based on assessments of individual risk and surveillance of AT levels, might help to optimize pregnancy outcomes in women with inherited AT deficiency. However, further research in this area is necessary, but it will be challenging due to the rareness of this serious disorder.

Conflict of interest statement

None declared.

Acknowledgements

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