Correlations between clinical and placental histopathological and immunohistochemical features in women with and without hereditary thrombophilia

JANINA GEORGIANA NACEA¹, IONELA ROTARU², MIHAELA NICULESCU³, RADU STĂNESCU³, NICOLAE CERNEA⁴, ANA-MARIA PĂTRAȘCU², LOREDANA ELENA STOICA⁵, ȘTEFANIA TUDORACHE⁴

¹PhD Student, Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania  
²Department of Hematology, University of Medicine and Pharmacy of Craiova, Romania  
³Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania  
⁴Department of Obstetrics and Gynecology, University of Medicine and Pharmacy of Craiova, Romania  
⁵Department of Dermatology, University of Medicine and Pharmacy of Craiova, Romania

Abstract

Aim: The primary objective of this study was to correlate hereditary thrombophilia (high- or low-risk) with specific placental histopathological (HP) and/or immunohistochemical (IHC) changes, for confirming/ruling out a possible linkage between these two biological parameters.  

Patients, Materials and Methods: We present a 3-year prospective study conducted between 2016 and 2019 that enrolled 90 women registered in two Clinics of Obstetrics and Gynecology in Craiova, Romania, with personal thrombotic and/or pathological obstetrical history. The HP and IHC analysis of the placenta was performed using monoclonal anti-cluster of differentiation 34 (CD34) antibody, anti-hypoxia-inducible factor-1 alpha (HIF-1α) and anti-endothelial nitric oxide synthase (eNOS) antibody. Results: There was a high incidence of all thrombophilia (TPh) mutations in Caucasian women with thrombotic and obstetrical complications. Among them, both HP and IHC examination revealed significant changes. These were more severe in the placentas of patients with homozygous Factor V Leiden (FVL) gene mutation and double heterozygous FVL/PII gene mutation. Multiple placental infarctions with massive fibrinoid necrosis and an increase in syncytial knots are common findings. In the same group, we found by means of IHC examination – intense positive HIF-1α and eNOS immunexpression, and low positive CD34 expression, especially in fibrinoid necrosis and thrombosis areas. We found no correlation between clinical, HP and IHC changes in patients with low-risk TPh or without TPh. Conclusions: Among patients with obstetric and thrombotic complications, there is a high prevalence of TPh. It appears that hypercoagulability reported in high-risk thrombophilia (HR-TPh) has major effects on placental tissue (fibrinoid necrosis, multiple thromboses, hypoxia and oxidative stress). Significant placental changes were found predominantly in women with HR-TPh. Strategies for TPh screening based on HP/IHC pattern would be, most probably, more cost-effective compared with the extended TPh testing offered in large populations. This way, a smaller number of patients will be tested and in this group a higher proportion of patients will be found as having HR-TPh mutations.

Keywords: thrombophilia, oxidative stress, hypoxia, placenta, thrombosis.

Introduction

The placenta is a vital organ involved in normal development and fetal growth, having a key role in maternal fetal exchanges of oxygen, nutrients, antibodies, hormones or other substances [1, 2]. In placental development, the blastocyst adheres to the decidua, leading to the differentiation of trophoblastic cells into cytotrophoblast and syncytiotrophoblast. The primary villi appear as tree branches, from which will develop the secondary and tertiary villi, establishing the early uteroplacental circulation [3]. Later, placental angiogenesis occur (the formation of new blood vessels from the existing ones), essential for optimal fetal development [4]. Disorders in placental implantation may result in maternal and fetal complications, typically manifesting in the first and the second trimester. Within such disorders, there are conventionally included: abnormal trophoblastic invasion, anomalies in angiogenesis and inappropriate remodeling of spiral arteries, thrombosis, and fibrin deposits [5]. A deficient perfusion may have as consequences: intrauterine growth restriction (IUGR), recurrent pregnancy loss (RPL) and preeclampsia (PE). These clinical features were also related with the presence of hereditary thrombophilia (TPh) [6].

The linkage between TPh and suboptimal perinatal outcome was increasingly discussed over the past 10 years [7]. The spectrum/severity of the pregnancy complications seems to be related with the type of coagulation factors mutations. The high-risk ones (HR-TPh) are considered: homozygous G1691A Factor V Leiden (FVL) gene mutation, homozygous G20210A prothrombin (PII) gene mutation, and double heterozygosity of FVL and PII gene mutation. These genetic mutations were reported to have, beside pregnancy-related complications [8], an increased risk (up to 17–34 times) for developing venous thromboembolism (VTE) also [8, 9]. Function of the thrombogenic potential, in low-risk thrombophilia (LR-TPh) are included the C677T and A1298C methylenetetrahydrofolate reductase (MTHFR) gene mutation and plasminogen activator inhibitor (PAI) gene mutation [10].
It has been demonstrated that TPh, especially if having high risk, is associated also with changes in placental parenchyma [11]. Although not pathognomonic, the most common placental lesions found in conventional staining techniques in women with TPh are: increased numbers of syncytial knots, intraparenchymal thrombosis, avascular villi, massive fibrinoid necrosis [12, 13].

We hypothesized that these placental lesions might have a linkage/mediating role in the clinical features in complicated pregnancy of women with TPh. Publications on this topic are scarce; therefore, the primary objective of this study was to correlate hereditary TPh (high- or low-risk) with some particular placental histopathological (HP) and/or immunohistochemical (IHC) changes in a selected case series (positive history for IUGR, RPL, PE, intrauterine fetal demise, VTE).

 Patients, Materials and Methods

We performed a prospective study between January 2017 and December 2018, enrolling patients registered in the Clinics of Obstetrics and Gynecology, “Filantropia” Municipal Hospital and Emergency County Hospital, in Craiova, Romania. For deciding the study group, we analyzed the observation sheets, the birth records and the electronic databases of the two Clinics, between January 2013 and December 2016. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova.

Inclusion criteria

Inclusion criteria, simultaneously met, were: 18 to 40 years old; Caucasian; non-smoker; previous pregnancy/pregnancies followed-up in one of the two Institutions involved; positive obstetrical history [more than three recurrent abortions, intrauterine fetal death (IUFD), IUGR, PE] and/or personal history of thrombosis; becoming pregnant again in the study period (2017–2019) and followed-up in the subsequent pregnancy in one of the two mentioned Hospitals.

In this study, IUGR was defined as fetal growth below the 10th percentile at any gestational age, mild PE – as blood pressure >140/90 mmHg, and severe PE – as blood pressure >160/110 mmHg in the third trimester of pregnancy.

The signed informed consent to participate, including the acceptance for HP analysis of the placenta, was mandatory for entering the study.

Exclusion criteria

Exclusion criteria were represented by: associated conditions – cardiac or kidney diseases, diabetes mellitus, systemic lupus erythematos (SLE), antiphospholipid syndrome (APS); prior initiated treatment for other diseases; patients already tested for TPh.

All women who met the inclusion criteria (see the workflow below – Figure 1) were then subjected to genetic tests for hereditary TPh. Also, their previous pregnancy’ placenta was analyzed following a standard protocol: conventional HP and IHC techniques. Due to the described selection criteria, we were able to include in this study 90 patients registered during the study period.

From the initial total group of 18 000 births, we found 3358 with a positive history (obstetric and/or thrombotic complications). 1562 did not meet the selection criteria, leaving a total of 668 potential cases for analysis. In 510 of them, we were able to find the placenta specimens. Among these, 120 patients had a new pregnancy between January 2017 and December 2018. Finally, 30 patients declined to participate in the study. Therefore, we ended-up having 90 women to meet the inclusion criteria and this was our final study group.

Figure 1 – Flow chart diagram. PE: Preeclampsia; IUGR: Intrauterine growth restriction; RPL: Recurrent pregnancy loss; VTE: Venous thromboembolism; SLE: Systemic lupus erythematos; APS: Antiphospholipid syndrome; TPh: Thrombophilia; HP: Histopathology; IHC: Immunohistochemistry.
**Histological analysis**

Placental fragments were obtained and subjected to fixation, embedded in paraffin and sectioned at 4 μm. The classical morphological Hematoxylin–Eosin (HE) staining was used to highlight the placental structure and pathological lesions, such as: vascular thrombosis, avascular villi, dilated stem villi accompanied by stasis, trophoblastic cells degeneration and calcification, intervillous hemorrhage, or syncytiotrophoblastic knots. Another morphological staining chosen was Goldner–Szekely (GS) trichrome, specific for the collagen fibers that stain in green. We used Periodic Acid Schiff–Hematoxylin (PAS–H), which identifies glycosaminoglycans.

The HP lesions were quantified as: absent (-), when the lesions were missing; (+), when lesions appeared focally; and increased (+++), when these appeared extensive. In this way, it is considered (+++) fibrinoid necrosis, when more than half of the chorionic villi is affected (a homogeneous extensive area of hyaline, eosinophilic material, where the collagen degenerates to a fibrin-like appearance), also single or multiple placental infarctions (one or more areas that affects the interstitial space, with partial or complete obstruction of the vessels), and (+++) syncytiotrophoblastic knots when appeared in more than 30% of chorionic villi.

**IHC analysis**

Considering that TPh is associated with placental vascular changes, we used for IHC analysis monoclonal anti-cluster of differentiation 34 (CD34) antibody (monoclonal mouse anti-human CD34 Class II, clone QBEnd 10, Dako, 1/50 dilution), which allows us to identify blood vessels into the placental tissue. We also use anti-endothelial nitric oxide synthase (eNOS) (rabbit polyclonal antibody, clone RB-9279-P1, NeoMarkers, 1/50 dilution) and anti-hypoxia-inducible factor-1 alpha (HIF-1α) (mouse monoclonal [mgc3] to HIF-1α, Ab 16066, Abcam, 1/600 dilution) for their important role in hypoxia.

IHC results were quantified as: negative (-), when there was no immunoexpression; low positive IHC (+); and intense positive IHC (+++), quantified according to the affected area. Depending on the TPh testing results, we divided the patients into two groups, as follows: Group A – patients with hereditary TPh, and Group B – patients without TPh. Group A was subsequently subdivided into HR-TPh and LR-TPh, as defined above.

**Statistical analysis**

All data were stored in Excel files for processing, according to the study design. Results were interpreted and statistical analyzed using Statistical Package for Social Science (SPSS) 20 software. Student’s t-test was used to compare quantitative data between groups and chi-square (χ²) test to determine if there were significant differences between the study groups. P-value <0.05 was considered significant.

For highlighting and significant statistics, we compared the history of maternal and fetal complications, placental HP and IHC changes and types of coagulation factor mutation [HR-TPh, LR-TPh and TPh (-)].

**Results**

The average age of women included in the study was 30.55 years, with a standard deviation of 5.13 years, the peak being found around 27–28 years old.

Group A [TPh (+)] consisted of 40 patients and Group B [TPh (-)] of 50 patients.

We present a global picture of results in Table 1.
As previous pregnancy complications: in Group A, seven patients had severe PE and eight IUGR. Thirty-nine women had a history of RPL (26 early pregnancy loss and 13 late pregnancy loss). Also, four patients had a history of fetal death.

In Group B [TPh (-)], we had eight patients with severe PE, seven with IUGR, and 41 (82%) had RPL. In this group, we had also a single case of fetal death.

Group A TPh genetic mutations’ distribution:

- HR-TPh (12.5%) – two patients with homozygous FVL gene, three patients with double heterozygous status FVL/PII gene mutation and one patient with heterozygous PII gene mutation, two with heterozygous PII gene mutation, four with homozygous MTHFR gene, three patients with double heterozygous status MTHFR/PAI gene mutation (Figure 2).

Below, we will describe the pattern of genetic mutations in relation with clinical complications in our study group.

Among the eight patients with IUGR, in the thrombophilic group (Group A) there were one patient with homozygous FVL gene mutation, three with double heterozygous FVL/PII, 23 with double hetero/homozygous MTHFR/PAI, one case presented heterozygous PII gene mutation, two were heterozygous for FVL gene and from Group B [TPh (-)], seven cases had this complication.

Among patients with PE, in Group A, one patient was homozygous for FVL, one patient double heterozygous FVL/PII, two cases presented double hetero/homozygous MTHFR/PAI, one patient heterozygous PII, two patients had heterozygous FVL and from Group B, eight patients had this complication. Among patients with RPL, one patient presented homozygous FVL gene mutation, four double heterozygous FVL/PII, 20 patients presented double hetero/homozygous MTHFR/PAI gene mutation, two PII heterozygous, and 10 cases had homozygous FVL. In Group B, 41 patients presented RPL. Another complication was IUFD present at one patient with homozygous FVL, two patients with double heterozygous FVL/PII, one patient with heterozygous FVL, and at one patient from Group B. VTE appeared only in one patient with double heterozygous FVL/PII status.

**HP exam**

In all patients with HR-TPh, we found multiple placental infarctions with vasoconstriction (obliterative endarteritis) and (+++) syncytial knots (Figure 3), but also (+++) fibrinoid necrosis with avascular villi, and impairment of normal architecture (Figure 4). Other placental lesions have been encountered in less than 40%, such as calcifications (Figure 5), edema, degeneration of the syncytiotrophoblastic layer, dilated villi, intervillous hemorrhage. In the LR-TPh group, 40% of cases had placental lesions, although less severe. In 60% of cases, the conventional HP exam revealed results compatible with normal placentation. (+) Fibrinoid necrosis has been found, without important modification of the villi architecture (Figure 5), single placental infarction, and focal (++) syncytial knots near to the ischemic areas only. Similar percentages (up to 30%) were found in TPh (-) patients (Group B), presenting one or more of the described lesions.

**Immunohistochemistry characteristics**

In Group A, we found as follows: in HR-TPh, a CD34 (+) and even CD34 (-) expression was found in large areas of fibrinoid necrosis, in all patients (Figure 6A). This type of immunoreactivity (mild or absent for CD34) was found also in 40% of cases with LR-TPh. In Group B [TPh (-)], a CD34 (+) expression was seen in 34% of patients.

In LR-TPh, CD34 (+++) immunoreactivity was found in smaller percentage (17.1%) of cases (Figure 6B). In Group B, CD34 (+++) patients appeared in 14% of cases (Figure 6C).

HIF-1α was positive among HR-TPh patients: we found HIF-1α (+++) expression in 100% of cases (Figure 7A). In LR-TPh, HIF-1α (+) immunoreactivity was observed in 14% of patients, in trophoblastic cells near to a thrombosis or minimal vascularization (Figure 7B). In patients from Group B [TPh (-)], HIF-1α (+++) expression was observed in a small number of cases (12%).

In HR-TPh group, eNOS (++) expression was observed in the placental tissue of all five patients (Figure 8A);
and eNOS (+) immunoexpression in patients with LR-TPh and in those with TPh (-) (<35%) (Figure 8B).

Current pregnancy outcome

In the next pregnancy, all patients diagnosed with HR-TPh were treated according to guidelines, with Enoxaparin in prophylactic dose, 4000 IU/24 h, maintained throughout pregnancy and six weeks postpartum. In these subsequent pregnancies, one only patient developed a mild form of PE that resulted in a good final maternal and neonatal outcome (late preterm iatrogenic birth at 35 weeks of amenorrhea). The rest of four patients having HR-TPh had an uneventful pregnancy evolution.

Two patients from the LR-TPh group developed IUGR and three patients were diagnosed with severe PE. In Group B, we had two cases of severe PE and four patients IUGR.

We had no IUFD or VTE in the whole group of subsequently followed-up pregnancies and in the puerperal period (the following six weeks).

Discussions

Our fundamental question was whether women with complicated pregnancy and TPh (+) have more specific HP and IHC changes compared with those TPh (-). The next question would be if these data have the potential to lower the TPh testing incidence in clinical practice.

We found an increased prevalence of all TPh mutations among patients with obstetric or thrombotic complications: 44.4% were diagnosed with this condition.

Significant association was observed between IUGR, PE and RPL on one hand and HR-TPh on the other, in these group all patients having at least one of these features that we defined as “positive history”. Many authors also highlighted an increased prevalence of hereditary TPh as a whole group (including the high and the low risk) in the population having complicated pregnancies, especially FVL and MTHFR genes mutations (present in up to 68% of patients with IUGR) [14–16].

Figure 3 – (A) Intermediate mature villi with pseudoangiomatous aspect and intervillous hemorrhage, increase syncytial knots; (B) Intermediate mature villi, villous fibrinoid necrosis, syncytial knots; (C) Stem villi with dilated blood vessels, thrombosis with incomplete occlusion, diffuse circumferential necrosis, trophoblastic degeneration; (D) Stem villi with dilated vessels some hyperemic, others with thrombosis, marginal fibrinoid necrosis, vita and syncytial knots. HE staining: (A) ×100; (C and D) ×200. PAS–H staining: (B) ×100. HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff.

Figure 4 – (A) Apoptotic cell fibrinoid mass, adjacent small mature intermediate villi (HE staining, ×200); (B) Stem villi with massive fibrinoid necrosis, micro-calcifications, trophoblastic degeneration (PAS–H staining, ×100); (C) Intense collagen deposits, perivillous fibrinoid necrosis, placental infarction (GS trichrome staining, ×100). HE: Hematoxylin–Eosin; PAS: Periodic Acid–Schiff; GS: Goldner–Szekely.
Figure 5 – (A) Syncytial knots, fibrinoid necrosis (HE staining, ×100); (B) Stem villi with fibrinoid necrosis associate with dilated stem villi, accompanied by stasis and pseudoangiomatous aspect – frequent intermediate mature villi with pseudoangiomatous aspect (GS trichrome staining, ×100); (C) Placental infarction with calcification area (PAS–H staining, ×100). HE: Hematoxylin–Eosin; GS: Goldner–Szekely; PAS: Periodic Acid–Schiff.

Figure 6 – (A) Chorial villi with endothelial cells low positive for CD34, negative CD34 immunoreaction in the fibrinoid necrosis area, in patients with HR-TPh; (B) Mature intermediate villi with positive CD34 peripheral immunoreactivity, focal syncytial knots at LR-TPh patients; (C) Villous tree with intense CD34 immunoreactivity in TPh (+) patients. Anti-CD34 antibody immunomarking: (A–C) ×100. CD34: Cluster of differentiation 34; HR-TPh: High-risk thrombophilia; LR-TPh: Low-risk thrombophilia.

Figure 7 – (A) Villous tree with highly positive HIF-1α immunoreaction at trophoblastic level, in patients with HR-TPh; (B) Stem villi with low positive HIF-1α immunostaining at trophoblastic level, and negative in degenerate trophoblastic zone, in patients with LR-TPh. Anti-HIF-1α antibody immunomarking: (A and B) ×100. HIF-1α: Hypoxia-inducible factor-1 alpha; HR-TPh: High-risk thrombophilia; LR-TPh: Low-risk thrombophilia.

Figure 8 – (A) Intensely positive eNOS immunoreaction at the elastic tissue of the arterial media, in patients with HR-TPh; (B) Villous infarction with intensely positive eNOS at the constricted arterial wall and weak eNOS immunoreactivity at the dilated lumen vessel, in patients with HR-TPh and LR-TPh. Anti-eNOS antibody immunomarking: (A and B) ×200. eNOS: Endothelial nitric oxide synthase; HR-TPh: High-risk thrombophilia; LR-TPh: Low-risk thrombophilia.
In the literature, spontaneous RPL and their association with mutations in coagulation factors were also repeatedly investigated. We were not able to confirm any correlations between RPL and TPh as a large group (we found no statistical significance – \( p>0.05 \)), although an increased incidence of TPh patients of late pregnancy loss was reported in previous publications [17–19]. It may be the case that we performed the study in two referral units, dealing with an approximately 25% of referred patients, more prone to have a significant obstetrical history. Another explanation could be the low number of cases enrolled.

The placenta may present multiple lesions that occur physiologically during gestation, without repercussions on the fetus. It has been suggested that patients with TPh have placental mediated complications due to vascular dysfunction followed by ischemic lesions, at least in extremely severe placental complications [13].

In TPh patients, significant changes, as severe ischemia, may occur due to multiple thrombosis in the placental parenchyma [20]. The issue of linkage between hypercoagulability present in TPh patients and the placental lesions at conventional HP exam is still debated. Beeksma et al., in 2012, in a study that included 65 placental samples from patients with IUGR could not demonstrate a link between placental lesions and TPh, suggesting that other factors may be involved in placental pathology [21]. In the present study, HP exam confirmed that placental lesions occurred with a much higher frequency in patients with hereditary TPh (Group A). We found a significant quantitative difference in placental lesions, compared with the specimens in the TPh (-) group. Moreover, the amount of the lesions involved was related not only to the presence or absence of TPh, but also on the type of mutation. We found no statistical significant differences between LR-TPh or TPh (-) and placental lesions (\( p<0.05 \)). Our results suggest that hypercoagulability reported in HR-TPh has effects on placental tissue, and all our five patients with HR-TPh and complicated pregnancy presented extensive HP changes.

Additionally, conventional stainings allowed the identification of fibrinoid necrosis areas and avascular villi in TPh (+) patients (Figure 4A), as reported before [5], most probable due to local ischemia. Lesions, although not pathognomonic, appear predominantly in patients with homozygous \( FVL \) and double heterozygous \( FVL/PI \) genes mutations. Multiple placental infarctions, the most common lesion (found in 44% of women with TPh), have been associated with multiple obstetric complications as reported by others [13]. Other authors have demonstrated that placental infarction was found in cases with PE, IUGR or IUFD [22], but a link between placental infarction and patients with or without TPh could not be demonstrated [23].

A striking difference was found between the HR-TPh and the other two large groups [LR-TPh and TPh (-)].

(++) Syncytial knots were found as aggregates of nuclei, developed on the trophoblastic surface, due to its apoptosis, as found by other authors [24, 25].

Fibrin formation and fibrinoid necrosis can occur because of hypoxia due to hypoperfusion or placental ischemia (Figure 3, A and B), and, on the other hand, due to the role of TPh in fibrin formation [26]. This is the hypothesis we were trying to demonstrate by applying specific IHC tests for angiogenesis (anti-CD34) and hypoxia (anti-HIF-1\( \alpha \) and anti-eNOS). Thus, we extended the protocol to check if a more specific relationship exists, between IHC techniques results and clotting factor mutations, as previously suggested [27].

**IHC exam**

Monoclonal anti-CD34 antibody was previously used to highlight the vessels density in placental tissue [28]. Considering that secondary hypoxia stimulate angiogenesis, we used monoclonal anti-CD34 antibody to clarify a theory previously formulated [29]. The secondary ischemia (following the vascular thrombosis) resulting in stimulation of pro-angiogenic growth factors and the formation of chionic villi neovascularization. This may be highlighted by the CD34 (++) expression in endothelial cells [26]. These changes were present also in placenta of patients with complicated pregnancies and LR-TPh and TPh (-) but the amount of the lesions were less extensive compared with the HR-TPh group (Figure 5A).

In the same placental tissue, areas of (++) fibrinoid necrosis with the absence of vascularization at this level, as well CD34 (+/-) immunoexpression were confirmed. These findings were present in all five patients with HR-TPh and in less than 40% patients with LR-TPh, also in TPh- patients.

Another pathological placenta feature we tried to compare was the presence of an intense oxidative stress associated with hypoxia in thrombophilic versus non-thrombophilic patients.

HIF-1\( \alpha \) is an important factor involved in hypoxia and may have two complementary functions. On the one hand, it can stimulate angiogenesis in conditions of newly installed hypoxia, by releasing growth factors [30]. On the other hand, if prolonged hypoxia present, it is insufficient and will induce apoptosis rather than neoformation. Thus, HIF-1\( \alpha \) presents both anti-apoptotic and pro-apoptotic effects [31].

Under the conditions of normal tissue oxygenation, HIF-1\( \alpha \) (+) or HIF-1\( \alpha \) (-) expression is found, due to its degradation, and HIF-1\( \alpha \) (++) expression if oxidative stress installed [32]. HIF-1\( \alpha \) (++) is also found in placental perfusion disorders secondary to local ischemia, or in obstetric complications, such as PE or IUGR [33]. It is usually found in hypoxic trophoblastic cells, having an important role in regulating oxygen homeostasis [34].

In our study, we have shown that patients with obstetric complications had a degree of hypoxia in the placenta tissue, but also more important data were obtained when the correlation was made between this marker and the clotting factor mutations. We highlighted that HR-TPh can induce additional oxidative stress as evidenced by HIF-1\( \alpha \) (++) expression in trophoblastic cells in the placenta specimens, being present in all five patients. In LR-TPh and TPh (-) patients, we found HIF-1\( \alpha \) (+) and HIF-1\( \alpha \) (-) alpha expression, with no significant HIF expression differences between these two groups. No statistical significance were observed between HIF expression and LR-TPh and TPh (-) (\( p>0.05 \)).
In placental perfusion disorders, oxidative stress is installed [35], which stimulates the antiangiogenic factors resulting in vascular constriction and thus hypoxia that may produce impaired fetal development [36]. Once hypoxia and vasoconstriction are installed [33], a release of nitric oxide appears at the vessel area, producing vascular dilation and smooth muscle cell relaxation [37].

The same interesting changes were observed in the blood vessels predominantly in patients from Group A, with proliferation and hypertrophy of smooth muscle cells, severe vasoconstriction, with eNOS (+++) expression.

In our study, we were able to see an important vasoconstriction in most patients in Group A with HR-TPh, secondary to oxidative stress and endothelial dysfunction. By eNOS staining, we noticed intense HR-TPh, secondary to oxidative stress and endothelial vasoconstriction in most patients in Group A with severe vasoconstriction, with eNOS (+++) expression.

Thus, we can state that the hypercoagulability confirmed in HR-TPh gene mutations favors the onset of certain placental reactions in the group that present unfavorable obstetrical outcome. The secondary hypoxia, and related HIF-1α (+++) and eNOS (+++) expression in these patients support this finding. eNOS (-)/eNOS (+) were more commonly seen in the LR-TPh group.

As strong points of our study, we mention a strictly standardized protocol in placental specimens’ analysis and in the pregnancy management. Moreover, we were not able to find in the literature other studies reporting for hereditary TPh, and the IHC of the placental tissue in complicated pregnancies, and this may be considered as a novelty.

In our view, the sample study may be considered still low, although we involved two hospital Units, we reviewed four years of retrospective data and two years of prospectively acquired data. Most probably, this is due to the HR-TPh prevalence in our Caucasian population and due to the high number of pregnant women already tested for TPh (1128 cases).

In the second pregnancy, the vast majority of complications were absent. This reality raises many questions about the general benefits of testing for TPh in both positive and negative history groups. Moreover, until recently, these tests were not subsidized by national health assurance system, and all tests were provided by the couples’ finances.

According to our preliminary results, LR-TPh appears to have no clinical, HP or IHC changes in addition to those seen in TPh (-) patients. These data should be verified in the future by designing other larger prospective studies.

Conclusions

Among patients with obstetric and thrombotic complications, there is a high prevalence of TPh. Using placental HP and IHC analysis with monoclonal antibodies, significant placental changes were found predominantly in women with HR-TPh. In our view, in patients with prior obstetric and/or thrombotic complications, placentas should be primary investigated by means of both HP and IHC techniques. We may speculate that this approach would be more cost-effective compared with the extended TPh testing in large populations. The next step would be testing for genetic mutations for TPh in the restricted group with significant HP/IHC pattern changes. Most probable, a smaller number of patients will be subjected to TPh screening and in this group, a higher proportion of patients will be found as having HR-TPh.

Conflict of interests

The authors declare that they have no conflict of interests.

References


25. Corresponding author
Ionela Rotaru, Lecturer, MD, PhD, Department of Hematology, Faculty of Medicine, University of Medicine and Pharmacy of Craiova, 2 Petru Rareș Street, 200349 Craiova, Dolj County, Romania; Phone +40740–045 276, e-mail: rodirot@yahoo.com

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