RÉSUMÉ
Étude immunohistochimique du facteur de von Willebrand en tant que marqueur de la dysfonction endothéliale de la zone utéro-placentaire et du myomètre dans la grossesse

Introduction. Pendant la grossesse, l’utérus subit un grand nombre de changements morphologiques. Les changements placentaires conduisent à un apport suffisant du foetus et à un processus normal d’accouchement. En particulier, la région utérine-placentaire apparaît quand l’ovule fécondé se fixe à l’utérus. Le myomètre augmente de taille et modifie la configuration spatiale.

L’objectif de l’étude était de développer la relative concentration immunohistochimique du facteur de von Willebrand dans les cellules endothéliales de différents types de vaisseaux de la région utérine-placentaire et du myomètre pendant la grossesse.
antigen thermal exposure (DACO); 2) differentiated histochemical fibrin and collagen Slinchenko’s staining; 3) hematoxylin-eosin staining. Digital images were analyzed with a computer program ImageJ (1.48v, W. Rasband, National Institute of Health, USA, 2015). We also calculated the arithmetic mean and its average error for optical density (computer program PAST 3.19, Ø.Hammer, 2018).

**Results.** The method was tested in 65 pregnant women during the cesarean section. The main results of the staining quantitative analysis in myometrium are: 1). Arterial type vessels 0.415±0.0029 units of optic density; 2). Venous type vessels 0.381±0.0024 units of optic density; 3). Microcirculatory system 0.375±0.0022 units of optic density. The main results of the staining quantitative analysis in uterine-placental area are: 1). Arterial type vessels 0.404±0.0027 units of optic density; 2). Venous type vessels 0.380±0.0024 units of optic density; 3). Microcirculatory system 0.373±0.0021 units of optic density.

**Conclusions.** Immunohistochemical study of von Willebrand’s factor allows investigation of the endothelial dysfunction in all types of vessels of both UPA and myometrium. This is very promising for the early detection of placental dysfunction and establishing of morphological preconditions for fetal insufficiency.

**Métodes.** Le matériel de biopsie a été obtenu des femmes enceintes pendant la césarienne au moyen de notre propre technique. Trois parties (centrale, périphérique, périphériques) de chaque biopsie ont été développées. Plusieurs sections de paraffine ont été colorées de trois manières différentes: 1) la coloration immunohistochimique du facteur de von Willebrand avec une exposition thermique antigénique; 2) la coloration histochimique de Slinchenko différenciée à la fibrine et au collagène; 3) la coloration à l’hématoxyline-eosine. Les images numériques ont été analysées avec un programme informatique ImageJ (1.48v, W. Rasband, Institut national de la santé, USA, 2015). Nous avons également calculé la moyenne arithmétique et son erreur moyenne pour densité optique (programme informatique PAST 3.19, Ø.Hammer, 2018).

**Résultats.** La méthode a été testée chez 65 femmes enceintes au cours de la césarienne. Les principaux résultats de l’analyse quantitative de la coloration dans le myomètre sont 1). Vaisseaux de type artériel 0.415±0.0029; 2) Vaisseaux de type veineux 0.381±0.0024; 3) Système de microcirculation 0.375±0.0022. Les principaux résultats de l’analyse quantitative de la coloration dans la région utérine-placentaire sont : 1). Vaisseaux de type artériel 0.404±0.0027; 2) Vaisseaux de type veineux 0.380±0.0024; 3) Système de microcirculation 0.373±0.0021.

**Conclusions.** L’étude immunohistochimique du facteur de von Willebrand permet l’étude du dysfonctionnement endothélial dans tous les types de vaisseaux de la région utérine-placentaire et du myomètre, ce qui est très prometteur pour la détection précoce du dysfonctionnement plaquettaire et l’établissement des conditions morphologiques préalables à l’insuffisance fœtale.

**Mots-clés:** région utérine-placentaire, myomètre, grossesse, facteur de von Willebrand.

**Abbreviations list:** uterine-placental area (UPA); von Willebrand’s factor (vWF), Ob – objective, Oc – ocular.
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THE OBJECTIVE OF THE STUDY was to assess the relative immunohistochemical concentration of the vWF in endothelial cells of different types of vessels of the UPA and myometrium in pregnancy.

MATERIALS AND METHODS

The biopsy material was obtained from 65 pregnant women during the cesarean section.

The investigation took place during 2014-2018 years at the Department of pathological anatomy of Higher Educational Establishment „Bukovinian State Medical University” (Chernivtsi, Ukraine). The biomat-erial for this research was taken on the basis of patient’s informed consent for taking the utero-pla-cental area and myometrium during the C-section in Kamyanets-Podilsky maternity hospital. Material harvesting and research design were approved by the Biomedical Ethics Committee of Bukovinian State Medical University.

Three parts (central, pericentral, peripheral) of each biopsy were fixed in 10% neutral buffered formalin solution for 22-24 hours, with further ethanol dehydration and paraffin embedding. Multiple paraffin sections (5 mkm) were stained in three ways: 1) immunohistochemical vWF staining with an antigen thermal exposure (DACO); 2) differentiated histochemical fibrin and collagen Slinchenko’s staining (Malory’s analogue techniques); 3) hematoxylin-eosin staining.

Optic density of specific staining was measured in relative units of optic density, by means of computer micro densitometry method (from 0 – absence of staining, absolute transparency; to 1 – maximal staining, absolute non-transparency) by means of computer program ImageJ (version 1.48v, free license, W.Rasband, National Institute of Health, USA, 2015). Optic density of staining was applied as a measure of immunohistochemical concentration

Arithmetic mean and its error were calculated. The groups were compared by means of bilateral unpaired Student’s criterion in the medium of computer program PAST 3.15 (free license), Ø. Hammer, 2018. Preliminary testing for the norm was made in samples by means of Shapiro-Wilki method. The differences were considered statistically significant if p≤0,05.

RESULTS

The positive staining of vWF was observed in endothelial cells in all types of blood vessels, but the color intensity was different. To establish a numeric value of the staining and to verify its difference in obedience to the type of vessel and the localization (myometrium or UPA), we defined the optical density (Table 1).

The biggest value of optical density was in arterial type vessels, and its value depended on localization – density was significantly higher in myometrium arteries (Fig. 1), smaller in venous type vessels and the smallest in endothelial cells of microcirculatory system (Fig. 2, 3). In these structures, optical density did not depend on localization.

In the lumen of the blood vessels we found vWF positive filamentous structures with varying color intensity, shape and size (Fig. 1). Those structures were located near the vessel wall or closed the vessel lumen completely or partially. Since those structures seems to be thrombi, we studied them by means of the Slinchenko’s histochemical technique, which is usually used for specific fibrin detection. Assumption about the nature of these objects was confirmed; the objects contained fibrin, and therefore were thrombi. However, it was noted that in the material from some women filamentous and formless intravascular structures were found more often in the case of using vWF staining than the Slinchenko’s staining. Thus, we can suggest that in thrombotic formation vWF accumulates in the vessels’ lumen earlier than fibrin.

The obtained results of the study of vWF staining are promising for the evaluation of endothelial dysfunction in different types of vessels, and for the arteries – in different localizations (myometrium or UPA).

During the study of the sections with immunohistochemical staining, in the lumens of the vessels the desquamated endothelial cells were found (Fig. 1,2,3). This finding is important for verification of

|                     | Myometrium (units of optic density) | UPA (units of optic density) |
|---------------------|------------------------------------|-------------------------------|
| Arterial type vessels | 0.415±0.0029*                      | 0.404±0.0027*                 |
| Venous type vessels  | 0.381±0.0024**                     | 0.380±0.0024**                |
| Microcirculatory system | 0.375±0.0022**                  | 0.373±0.0021**                |

* – two samples are significantly different, p<0,05
** – two samples are NOT significantly different
Figure 1. Arterial type vessels Slinchenko’s staining (on the left) and immunohistochemical vWF (on the right) staining. Ob 40×. Oc 10×

Figure 2. Venous type vessels Slinchenko’s staining (on the left) and immunohistochemical vWF (on the right) staining. Ob 10×. Oc 10×

Figure 3. Microcirculatory vessels Slinchenko’s staining (on the left) and immunohistochemical vWF (on the right) staining. Ob 40×. Oc 10×
these cells, because in a few minutes after the desqua-
mation they are going through the process of anoikis
and become round-shaped. After that, the morpho-
logical verification of such endothelial cells is mostly
impossible. The detection of desquamated endothel-
ial cells witnesses the endothelial dysfunction and
indicates the gross damage of the intima of the blood
vessel. Therefore, desquamated endothelial cells
can be verified by means of detection of vWF.

Another interesting fact was that the groups of
erythrocytes in the lumen of the venous vessels and
the microcirculatory vessels were stained with the
same method, too (Fig. 2,3). According to our assump-
tion, this fact can be an artifact, caused by the insuffi-
cient neutralization of erythrocytes peroxidase during
the immunohistochemical method. We repeated the
study of serial sections of the same myometrium and
UPA biopsy with more prolonged neutralization of
endoperoxidase and with a double change of neutral-
izing reagent. The result was the same.

Due to the location of these changes (venous ves-
sels, microcirculatory channel) and staining of only
the periphery of erythrocytes, it was concluded that it
was caused by the adhesion of vWF on erythrocytes.
This can mean the early stage of formation of intra-
vascular blood clots in vessels with a slow blood flow.

DISCUSSION

The vWF is a plasma glycoprotein, which is one
of the key components of hemostasis, contributing to
the attachment of blood platelets to damaged areas in
the blood vessels\(^{18}\). Specific vWF receptors are found
both in the plate's membrane and in the sub-endo-
thelium. Since the endothelial cells synthesize vWF
more than necessary, this excess is stored inside the
endothelial cells in special organelles called Weibel-
Palade bodies. They excrete vWF after the stimula-
tion of endothelial cells\(^{11,12}\).

We drew attention to the lack of information in
the scientific literature describing the results of the
vWF study in the vessels of the utero-placental area
and myometrium in both physiological and compli-
cated pregnancy. In this aspect, we consider to be in-
teresting the study of pregnancy with both blood or
cardiovascular diseases and pathology of cytotropho-
blastic invasion in the background. For example, iron
deficiency anemia leads to low levels of serum iron,
changes in red blood cells – hypochromia, microcy-
tosis, spherocytosis, echinocytosis and others con-
tribute to the growth of the intensity of free radical
processes, limited proteolysis etc, and in the intersti-
tial spaces of the placenta – increased hemolysis with
the release of erythrocytic factors that are involved in
fibrin and fibrinogen formation. The pathology of
the „mother-placenta-fetus” system may be caused by
gestational immaturity of the utero-placental complex,
complete substitution of the fibrinoid walls of the
spiral arteries, hypoperfusion of the interstitial space,
violet angiogenesis or hyperplasia of the venous ves-
sels of the utero-placental area. All these factors create
prerequisites for increasing endothelial dysfunction
both in the vessels of the utero-placental complex, and
in the whole organism of the pregnant woman\(^{1,9,12}\).

As a result of this study, we found a lower con-
centration of vWF in endothelial cells of the micro-
circulatory system, higher concentration of vWF in
the endothelial cells of the venous type vessels of the
utero-placental area and myometrium (approximately
the same amount) and the highest – in arterial type
vessels (significantly higher in myometrium) (Table 1).

The prospect of further research is related to
the testing of immunohistochemical techniques on
the vWF in the UPA and the myometrium of preg-
nant women with various types of pathology of preg-
nancy, and first of all, the utero-placental form of
placental insufficiency.

CONCLUSIONS

Immunohistochemical study of vWF allows in-
vestigation of the endothelial dysfunction in all types
of vessels of both UPA and myometrium. This can
be very promising for the early detection of placental
dysfunction and establishing of morphological pre-
conditions for fetal insufficiency.

We established that immunohistochemical vWF
staining on the basis of observation of pregnancy
with signs of utero-placental hemocycling pathology
reveals early thrombotic formation, blood convec-
tion and endothelium damage in myometrium and
utero-placental area more efficiently than the classic
methods of fibrin staining.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding
this article“

„The authors declare that all the procedures and ex-
periments of this study respect the ethical standards in the
Helsinki Declaration of 1975, as revised in 2008(5), as
well as the national law. Informed consent was obtained
from all the patients included in the study

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