Chapter 6

Endothelial Dysfunction as a Consequence of Endothelial Glycocalyx Damage: A Role in the Pathogenesis of Preeclampsia

Marina M. Ziganshina, Ekaterina L. Yarotskaya, Nicolai V. Bovin and Gennady T. Sukhikh

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75043

Abstract

The endothelial glycocalyx is an intravascular compartment which consists of carbohydrate part of membrane glycoconjugates, free proteoglycans and associated proteins. It is thought to play an important role in the vascular tone regulation, vascular permeability and thromboresistance. It was suggested that the leading cause of endothelial dysfunction in various cardiovascular, inflammatory, and kidney diseases is the damage of the endothelial glycocalyx. This review presents the changes in the composition and structure of the endothelial glycocalyx in the settings of damage and under systemic inflammatory response, and the impact of these changes on the functions of endothelial cells and intercellular contacts, mediating the interaction of endothelium and the immune cells. The second issue, discussed in this article is a possible role of endothelial glycocalyx in the pathogenesis of preeclampsia—a complication of pregnancy associated with hypertension, proteinuria and edema. The reviewed data contribute a new insight in the endothelial dysfunction pathogenesis.

Keywords: glycocalyx, endothelial glycome, endothelial dysfunction, glycobiology of inflammation, pregnancy, preeclampsia

1. Introduction

Preeclampsia (PE) is one of the main problems of modern obstetrics. PE develops in 2–9% of all pregnancies; it is the second most frequent cause of maternal morbidity and one of the leading causes of neonatal morbidity and mortality. PE is now regarded as a syndrome

which is caused by disrupted adaptation to pregnancy and manifests with the development of complex, multiorganic and polysystemic insufficiency with clinical signs appearing after the 20th week of gestation [1, 2]. Despite of vigorous research in this area, pathogenesis of PE is still not clear. However, it is well known that the key factors of PE are immune system hyperactivation and the following excessive systemic inflammatory response (SIR), which initiate endothelium activation and cause endothelial dysfunction in cases of early onset and complicated course of the disease [3].

Inflammatory response is accompanied by cell phenotype transformation (formation of activation cell status), leading to the generation of "danger" signals (generated from products of trauma, ischemia, necrosis or oxidative stress) [4, 5], which are recognizable by the immune system. It was found that the composition of endothelial glycocalyx (eGC) changed under excessive inflammatory response. Hypoglycosylated structures which may be perceived by immune system as neoantigens, appear on the membrane of endothelial cells; also, antigens which are normally covert become apparent [6]. These events may promote autotolerance disruption and cause production of autoreactive antibodies damaging endothelial cells. In this regard, in this chapter a special attention is paid to eGC—functional layer of endothelial cells, which mediates all endothelial functions. Much evidence that under SIR, the alterations of eGC are associated with changes of cardiovascular system hemodynamics, vascular tone regulation, vascular permeability [7]—the main vectors of pathophysiological disorders in PE, and that alterations affect endothelial autoimmune phenotype formation, allow to assume that eGC may be one of the main targets of PE.

2. Endothelium: its role in homeostasis and in pathology

Vascular endothelium is a metabolically active neuroendocrine organ, which is spread in all tissues. The main functions of endothelium are: expression of receptor molecules, synthesis and secretion of biologically active molecules, vascular tone control, vascular permeability and new vessels formation, transportation of blood cells and soluble factors; homeostasis balance, participation in innate and adaptive immunity [8–10].

Supporting homeostasis, the endothelium is also subject to damage by factors, which cause endothelium pathology. Multiorganic dysfunction due to long-lasting activation under the effects of damaging factors lead to severe consequences.

Recent studies show that the homeostatic control over the cardiovascular and other systems is, among others, exerted by eGC, the outer above-membrane endothelium layer, which is formed by the sugar chains of transmembrane glycoconjugates and the associated not-anchored proteoglycans [7]. However, there is limited data on eGC composition and its alterations under inflammatory and other pathological conditions.

2.1. Endothelial glycocalyx structure and composition

Endothelium surface layer is located on the luminal surface of the endothelium (endothelial surface layer—ESL). It is formed by the glycoproteins, proteoglycans and glycosphingolipids

that are anchored in the membrane, as well as by secretory proteoglycans and glycosaminoglycans (GAGs), that are not anchored and are inter-connected by non-covalent interactions [11–13]. Their carbohydrate part contains a large amount of sialo and sulpho residues, forming overall negative charge of the endothelial cell surface. The outer segment of this layer (spreading out toward the vascular lumen), formed by the carbohydrate part of glycoconjugates, is a polysaccharide gel—eGC [14], with thickness ranging 2–4.5 μm [15] in different departments of the vascular system.

The base of the eGC is formed by carbohydrate-protein conjugates—transmembrane and secretory proteins; their carbohydrate part is represented by both short (2–15 monosaccharide residues) branched oligosaccharides, often decorated with sialic acid and sulfate (in glycoproteins), and by high-molecular glycans, often ending with highly sulfated residues (in proteoglycans) [16]. The glycoproteins can contain N-linked (Asn-linked) and/or O-linked (Ser/Thr-linked) glycans of variable length and composition. Complex hybrid and high-mannose glycans are usually present in the glycoproteins [17]. The main glycoproteins of endothelial cells are cell adhesion molecules (selectins, integrins, immunoglobulin superfamily molecules, endothelial mucins and addressins) which provide homing, migration and interaction between cells in different processes, and secretory molecules associated with eGC, participating in vascular homeostasis support, fibrinolysis and coagulation (thrombomodulin, von Willebrand factor (vWF)), antithrombin III, etc.). These molecules expression depends on factors, altering endothelium activation [16]. Under inflammatory response, the glycans modification occurs, leading to alteration of intercellular contacts, hemostasis and blood rheology. Biochemical eGC composition (the main structural and associated molecules) is presented in **Tables 1** and **2** (parts I and II).

It was found that the carbohydrate part is crucially important for glycoprotein function. N-linked glycans, particularly high-mannose chains, determine specific interactions of different molecules from the intercellular adhesion molecule (ICAM) family with the receptors [17]. N-glycans of the junctional adhesion molecule-A (JAM-A) regulate leukocyte adhesion and lymphocyte function-associated antigen-1 (LFA-1) binding [22]. Platelet/endothelial cell adhesion molecule-1 (PECAM-1 or CD31), a membrane highly glycosylated protein (~30% of molecular mass), has N-linked glycans represented by neutral and sialylated glycans [51, 52]. E-selectin is heavily glycosylated protein with hybrid/complex type N-linked oligosaccharides [53]. Cadherin of the vascular endothelium (VE-cadherin, CD144)—is the main transmembrane protein of adhesion contacts; its carbohydrate part is presented mainly by sialylated biantennary N-glycans of a complex type, and sialylated hybride N-chains (~40 and 28% of all identified glycans, respectively). Branched tri- and tetraantennary N-glycans, as well as N-glycans of high-mannose type are represented in smaller quantity in N-glycans of VE-cadherin [21, 54]. In the presence of anti-inflammatory factors (such as tumor necrosis factor-α, TNF-α) the quantity of glycans ending with α2,6-sialic acid residues and fucose-α1,2-galactose-β1,4-N-acetylglucosamine increases, as well as the expression of N-glycans of high-mannose and hybrid type, which mediate intercellular contacts of monocytes with endothelium in the rolling and adhesion, particularly at the intercellular connections sites [55].

Hemostasis controlling proteins associated with outer eGC are also highly-glycosylated. VWF is a key component for maintenance of normal hemostasis, acting as the carrier protein of

Group	Members	Comments	References
Adhesion molecules	E-selectin	Contains 11 potential N-glycosylation site	[13]
	P-selectin	Contains 9 potential N-glycosylation sites	[13]
	Integrins: α1β1, α2β1, α3β1, α5β1, α6β1, α8β1, α9β1, αVβ1, αVβ3, α6β4, αVβ5	N-linked glycans	[14, 15]
	VE-cadherin	Contains 7 potential N-glycosylation sites	[16]
	JAM-1	Contains 1 N-glycosylation site	[17]
	JAM-2	Contains 2 N-glycosylation sites	[18]
	JAM-3	Contains 2 N-glycosylation sites	[19]
	ICAM-1	Contains 8 N-glycosylation sites	[13]
	ICAM-2	Contains 6 N-glycosylation sites	[20]
	VCAM-1	Contains 6 N-glycosylation sites	[13]
	PECAM-1	Contains 9 N-glycosylation sites	[13]
	ClyCAM-1	Mucin, containing predominantly O-linked carbohydrate chains (T-antigen and 6′ sulfated sialyl-Lewis-X)	[21, 22]
	CD34	Mucin, O-glycosylation sites are more abundant than N-glycosylation sites	[23]
	MadCAM-1	Mucin; contain O-linked glycans (SLe^x)	[24]
Coagulation and fibrinolysis regulators	Von Willebrand factor	Contains at least 10 potential N- and 10 O-glycosylation sites	[25, 26]
	Thrombomodulin	Contains at least 4 N- and 1 O-glycosylation sites	[27–32]
	Antithrombin III	Contains 4 potential N-glycosylation sites	[33, 34]
	Heparin cofactor II	Contains 3 potential N-glycosylation sites	[35]

MadCAM-1, mucosal addressin cell adhesion molecule-1; JAM-1, junctional adhesion molecule-1; JAM-2, junctional adhesion molecule-2; JAM-3, junctional adhesion molecule-3; ICAM-1, inter-cellular adhesion molecule-1; ICAM-2, inter-cellular adhesion molecule-2; VCAM-1, vascular cell adhesion molecule-1; PECAM-1, platelet/endothelial cell adhesion molecule-1; SLe^x, sialyl-Lewis-X.

Table 1. Biochemical composition of endothelial glycocalyx—main components (part I: glycoproteins).

the coagulant Factor VIII and mediating platelet adhesion at the sites of vascular injury [31]. VWF is heavily glycosylated by N- and O-linked oligosaccharides, and glycosylation affects many of its functions [30]. Antithrombin is a major inhibitor of the blood coagulation cascade.

Group	Members	Number/type of GAG-chains linked	Comments	Ref
Glycosaminoglycans	HA	—	Anionic, nonsulfated glycosaminoglycan; structural unit of HA is a repeating disaccharide consisting of β-D-glucuronic acid and β-N-acetyl-D-glucosamine; contains no core protein	[9]
	HS	—	The most common disaccharide unit within HS is composed of a mono-sulfated β-glucuronic acid linked to tri-sulfated α-N-acetylglucosamine	[36]
	CS	—	CS is a linear acidic polysaccharide, composed of repeating disaccharide units of β-glucuronic acid and β-N-acetyl-D-galactosamine and modified with sulfate residues at different positions	[37]
	DS	—	Backbone of DS chains is a linear polymer composed of repeating disaccharide units of α-iduronic acid and β-N-acetyl-D-galactosamine. These sugar residues can be modified by ester sulfate at various positions	[38]
	KS	—	Basic repeating disaccharide unit within keratan sulfate is units of β-D-galactose and β-N-acetyl-D-galactosamine	[39]
Proteoglycans (extracellularly secreted)	Perlecan	3/HS,CS	A large basement membrane heparan sulfate proteoglycan; protein core of approximately 500 kDa	[40]
	Versican	10-30/CS,DS	Large aggregating chondroitin sulfate proteoglycan, core protein (at >350 kDa)	[41, 42]
	Endocan	1/DS	Is a DSPG, small proteoglycan molecules (20 kDa) with a single DS chain; DS of endocan consists of about 32 disaccharide units	[43]
	Decorin	1/CS,DS	A prototype small leucine-rich proteoglycan (40 kDa); it has N-terminal attachment site for a single GAG chain of chondroitin or dermatan sulfate	[44]
	Biglycan	2/CS,DS	small leucine-rich proteoglycan (42 kDa protein core)	[45, 46]
	Mimecan	2–3/KS	Small leucine-rich proteoglycan; (12–34 kDa protein core)	[47, 48]
Proteoglycans (associated with the cell surface)	Syndecans	5/HS,CS	Transmembrane proteoglycans Family of HSPGs, the syndecan protein family has four members. Core protein of all glypicans is ranging between 198 to 346 kDa	[49]

Group	Members	Number/type of GAG-chains linked	Comments	Ref
	Glypicans	3/HS,CS	GPI-anchored proteoglycans Family of HSPGs The glypican protein family has six members core protein of all glypicans is similar in size, approximately ranging between 60 and 70 kDa	[50]

GAG, glycosaminoglycan; HA, hyaluronan; HS, heparan sulfate; CS, chondroitin sulfate; DS, dermatan sulfate; KS, keratan sulfate; DSPG, dermatan sulfate proteoglycans; HSPGs, heparan sulfate proteoglycans.

Table 2. Biochemical composition of endothelial glycocalyx—main components (part II: glycosaminoglycans and proteoglycans).

Two isoforms exist in the circulation, α-antithrombin and β-antithrombin, which differ in the glycosylation of the polypeptide chain; β-antithrombin lacks the carbohydrate present at Asn135 in α-antithrombin. Of the two forms, β-antithrombin has the higher affinity for heparin due to the conformational change that occurs upon heparin binding being sterically hindered by the presence of the additional bulky glycan in α-antithrombin [56]. The carbohydrate structures of heparin cofactor II (member of serpin superfamily) circulating in blood are complex-type biantennary and triantennary chains in a ratio of 6:1 with the galactose being >90% sialylated with α2-6-linked N-acetylneuraminic acid. About 50% of the triantennary structures contain one sialyl Le^x motif (SLe^x) [40]. Thrombomodulin (TM) is an endothelial cell surface glycoprotein (contains N- and O-linked glycans) that directly inhibits the procoagulant activities of thrombin and the TM-thrombin complex accelerates the thrombin catalyzed activation of protein C. Moreover, the GAG O-linked chains of TM contained chondroitin-4-sulfate and dermatan sulfate, which were repeated approximately 30 times. Soluble TM in urine has no GAG chain which could promote its anticoagulant activities. Studies of the rabbit recombinant TM have shown that addition of a GAG chain may increase its anticoagulant function [33, 34].

Endothelial mucins (CD34; glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1); mucosal addressin cell adhesion molecule-1 (MadCAM-1)) contact leukocytes by their binding to L-selectin. This interaction facilitates leukocytes transportations from blood to lymphoid organs and inflamed tissues [28]. Major capping group in GlyCAM-1, CD34 and MadCAM-1 is the sulfated SLe^x [27, 28, 57]. For example, CD34 functions as a L-selectin ligand mediating lymphocyte extravasation only when properly glycosylated to express a sulfated carbohydrate epitope. CD34 can exist in 2 glycoforms: the L-selectin-binding (L-B-CD34) and non-binding (L-NB-CD34) glycoforms. L-B-CD34 is relatively minor compared with L-NB-CD34 and represents less than 10% of total CD34. It has been shown, that a minor glycoform of CD34 carries relatively abundant 6-sulfo SLe^x epitopes on O-glycans that are important for its recognition by L-selectin [28].

The eGC mostly consists of proteoglycans—highly glycosylated proteins (glycans account for 90–95% of the molecular mass); GAGs branches form their carbohydrate part. There are

five types of GAG chains: heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronan (hyaluronic acid, HA). They are linear polymers of disaccharides with variable lengths that are modified by sulfation and/or (de)acetylation to a variable extent [15]. In the human body the GAGs are present in a protein bound form (i.e., in proteoglycans composition) and do not exist in a free form, except for HA. Besides playing structuring and supporting roles, proteoglycans are involved in cell signaling, regulation of cell proliferation, adhesion, migration, differentiation [55]. Key eGC glycans are heparan sulfate proteoglycans (HSPGs), which compose about 50–90% of the total amount of proteoglycans present in the eGC, and HA—the main supporting glycan [14, 15]. Main proteoglycans of the eGC and their characteristics are given in **Table 2** (part II).

Glycosphingolipids (GSLs), a class of ceramide-based glycolipids, are also a significant part of eGC. Glycosphingolipids are subclassified as neutral (no charged sugars or ionic groups), sialylated (gangliosides), or sulfated [58]. GSLs cluster with cholesterol in cell membranes to form GSL-enriched lipid raft [59]. Cultured human umbilical vein endothelial cells (HUVEC) appeared to contain complex lacto and globo series compounds (lactosylceramide, Gb_3Cer and Gb_4Cer), but the most abundant neutral GSL is lactosylceramide (LacCer, CDw17) [60]. LacCer can bind to various microorganisms, is highly expressed on the plasma membranes of human phagocytes, and forms lipid rafts containing the Src family tyrosine kinase Lyn. LacCer-enriched lipid rafts mediate immunological and inflammatory reactions, including superoxide generation, chemotaxis, and non-opsonic phagocytosis [61, 62]. Therefore, LacCer-enriched membrane microdomains are thought to function as pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) expressed on microorganisms. LacCer also serves as a signal transduction molecule for functions mediated by CD11b/CD18-integrin as well as being associated with several key cellular processes [63]. Endothelium activation by pro-inflammatory cytokines, particularly by TNF-α, affect the Gb_3Cer and Gb_4Cer [64] expression; interferon gamma (IFNγ) has a striking effect on the surface expression of GSLs; IL-1 increases the cell content of neutral and acidic GSLs but does not alter their surface expression [55]. Cytokines TNF-α and IL-1 can potentiate the toxic effect of verocytotoxin (Shiga-like toxin-produced by *Escherichia coli* and the main cause of hemolytic uremic syndrome) to human endothelial cells by inducting an increase in the Gb_3Cer synthesis in these cells [65], because Gb_3Cer (CD77) binds to the verocytotoxin and injures human endothelial cells [66].

Acidic GSLs of human endothelial cells are: monosialoganglioside or GM3—the major ganglioside of endothelial cells, and it constitutes about 90% of the whole ganglioside fraction [67], and sulfoglucuronyl paragloboside (SGPG), a minor GSL in endothelial cells, is a ligand for L-selectin [55]. Although GIyCAM-1 and CD34 constitute the major L-selectin ligand on venous endothelium, endothelial SLe^x gangliosides may also play a role, since L-selectin can also bind SLe^x GSLs under physiologic flow conditions [68].

2.2. Functions of the endothelial glycocalyx

The eGC is considered as an intravascular compartment which has various functions.

First, eGC mediates the endothelial mechanotransduction of shear stress and performs regulation of shear stress-induced nitric oxide (NO) production [69]. This is provided by the

impact of tangential stress of blood flow shift primarily to eGC; the latter accepts and scatters the load, created by fluid shear stress. Local spin moment, created by fluid shear stress, affects the proteoglycans chains, and further—the core proteins (syndecans and glypicans), causing actin cytoskeleton reorganization and transmission of the signal into the cell and the cell nucleus [70, 71]. The study of Fu and Tarbell (2013) aimed to determine the eGC role in mechanosensing and transduction, and measured the flow-induced production of NO *in vitro* [7]. It was found that compared to static conditions, the application of steady flow shear stress rapidly increased NO production from the baseline in bovine aortic endothelial cells. Enzymatic treatment of the key components of eGC (HS, HA) completely blocked flow-induced NO production without affecting receptor-mediated NO production, suggesting that the eGC has a direct effect on the NO production machinery [7]. Therefore, the eGC under physiological conditions (intact eGC) transforms hemodynamic effect into cell biochemical signals, which regulate the vascular tone.

Second, the negatively charged eGC forms a polyanionic hydrated mesh on the surface of endothelial cells, which acts as a selective permeability electrostatic barrier for plasma cells and proteins and serves as a selective permeability [72]. According to Salmon and Satchell, in both continuous and fenestrated microvessels, this eGC is acting as an integral component of the multilayered barrier provided by the walls of these microvessels (i.e., acting in concert with clefts or fenestrae across endothelial cell layers, basement membranes and pericytes) [73]. Dysfunction of any of these capillary wall components, including the eGC, can disrupt normal microvascular permeability. Disruption of eGC manifests with increased systemic microvascular permeability and albuminuria in the glomerulus [73]. Evidence from the experiments on Munich-Wistar-Fromter (MWF) rats, used as a model of spontaneous albuminuric chronic kidney disease (CKD), confirm that loss of eGC could contribute to both renal and systemic vascular dysfunction in proteinuric CKD [74]. Also, in the 5/6-nephrecomized rats model with CKD a significant decrease in eGC thickness and stiffness in the blood explants of aorta endothelial cell isolated from CKD rats was demonstrated [75]. An increase of the levels of the two major components of the eGC, namely syndecan-1 (Syn-1) and HA, in the blood of patients with CKD indicated the disease progression and correlated tightly with plasma markers of endothelial dysfunction such as soluble fms-like tyrosine kinase-1 (sFlt-1), soluble vascular adhesion molecule-1 (sVCAM-1), vWF and angiopoietin-2 [75]. The study of experimental eGC degradation in mice induced by long-term hyaluronidase infusion, including evaluation of the eGC thickness and composition by immunohistochemical methods and by transmission electron microscopy for complete and integral assessment of glomerular albumin passage, showed that glomerular fenestrae were filled with dense negatively charged polysaccharide structures that were largely removed in the presence of circulating hyaluronidase, leaving the polysaccharide surfaces of other glomerular cells intact [76]. Thus, HA is a key component of the glomerular endothelial protein permeability barrier; reduction of the HA facilitates albumin passage across the endothelial layer and the glomerular basement membrane toward the epithelial compartment [76].

Regulation of selective permeability by eGC, and the role of its separate components in this, is still subject of discussion. According to Lennon and Singleton, the HA plays key role in supporting endothelial barrier function [77]. HA maintains vascular integrity through eGC

modulation, caveolin-enriched microdomain regulation and interaction with endothelial HA binding proteins. Certain disease states, especially accompanied by SIR, increase hyaluronidase activity and reactive oxygen species (ROS) generation which break down high molecular weight HA to low molecular weight fragments causing damage to the eGC. Further, these HA fragments can activate specific HA binding proteins upregulated in vascular disease to promote actin cytoskeletal reorganization and inhibition of endothelial cell–cell contacts [77]. A glycocalyx-junction-break model, described by Curry and Adamson summarizes multiple studies and the role of the eGC in vascular permeability regulation [78]. According to this model, the layered structure of the endothelial barrier requires continuous activation of signaling pathways regulated by sphingosine-1-phosphate (S1P) and intracellular cAMP. These pathways modulate the adherens junction (*zonula adherens*), continuity of tight junction strands, and the balance of synthesis and degradation of eGC components [78].

Third, the eGC forms anti-inflammatory and anti-adhesive barrier at the endothelial cells. Vascular protection via inhibition of coagulation and leukocyte adhesion is provided by maintenance of the composition permanence and balance of degradation under the impact of stress shift and synthesis of eGC components [73, 79]. Total negative charge, formed by carbohydrate residues of the glycoconjugates chains on cell surface, prevents adhesive interactions of blood cells with vascular wall, biologically active molecules with anti-thrombotic action, while eGC-associated molecules provide hemostasis [80, 81]. Also eGC plays a structural role, impeding adhesion by covering adhesion molecules on the surface of the cell and by creating steric hindrance, making leukocyte binding more challenging [82]. Under the effect of damaging factors, the structure and composition of eGC change, its thickness may reduce significantly, and carbohydrate residues, normally covert and masked, become apparent. Main damaging factors, affecting the eGC *in vivo,* are: inflammation, hyperglycemia, endotoxemia, septic shock, oxidized low-density lipoproteins, cytokines, natriuretic peptides, abnormal shift stress and damage due to ischemia-reperfusion [79]. Shedding of eGC components in response to cytokines and chemoattractants occurs in all compartments of microvasculature: arterioles [83], capillaries [83, 84] and venules [84–86].

According to Lipowsky, the studies of leukocytic-endothelial adhesion in response to chemoattractants and cytokines, and shedding of constituents of the eGC, suggest that activation of extracellular proteases (matrix metalloproteases, MMPs) play a role in mediating the dynamics of leukocytes adhesion in response to inflammatory and ischemic stimuli [79]. Inhibition of MMP activation with sub-antimicrobial doses of doxycycline, or zinc chelators, have also inhibited leukocytes adhesion and shedding of glycans from the endothelial cells surface in response to the chemoattractant. Experiments by McDonald et al. have confirmed that under the enzymatic degradation of eGC with heparinase, endothelial cells developed a pro-inflammatory phenotype when exposed to uniform steady shear stress leading to an increase in leukocyte adhesion [82]. The results show an up-regulation of ICAM-1 (expression increases in 3 times) with degradation compared to non-degraded controls, and attribute this effect to a down-regulation in nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity in response to flow; this suggests that eGC is not solely a physical barrier to adhesion but rather plays an important role in governing the phenotype of endothelial cells, a key determinant in leukocyte adhesion [82]. Other mechanisms also contribute to the initiation

of lymphocytes adhesion to the endothelial cells after reduction of eGC layer: decrease of NO production, which is capable to inhibit leukocyte-endothelial cell adhesion [87]; appearance of eGC fragments, (such as low-molecular-weight HA), which show their pro-inflammatory properties, affecting the maturity of dendritic cells and stimulating them to produce cytokines [14, 88]; and exposure and synthesis under inflammatory response of hypoglycosylated structures, which interact with cell adhesion molecules of leukocytes [18, 89].

Modulation of eGC structure under effects of damaging factors, including inflammation, shows a thromboresistance loss [90, 91]. This occurs due to destabilization of heparin sulfate chains, the binding sites for coagulation inhibitor factors (antithrombin-III, the protein C system, and tissue factor pathway inhibition); this leads to a reduction of their local concentration at the vascular wall. In turn, a concentration gradient of protective and regulative molecules, associated with eGC (albumin, fibrinogen, orosomucoid, extracellular superoxide dismutase, fibronectin, vitronectin, collagens, thrombospondin-1 and other), and of growth factors (fibroblast growth factors, vascular endothelial growth factors, transforming growth factor-β, platelet-derived growth factors) is also decreased, facilitating pathological processes in blood vessels [80].

Therefore, the eGC is a labile structure; its composition changes under effects of damaging factors. This determines development of pathophysiological processes of endothelium activation/dysfunction with loss of vascular tone regulation, hemostasis and barrier function. Endothelium activation/dysfunction is induced by inflammation and accompanies it, thus forming a vicious cycle, which can be overcome only under normal immune system functioning. Inflammatory response of various degree accompanies not only pathologic processes, it is also observed under physiological conditions, for example, a pro-inflammatory background is shown at certain periods of normal pregnancy.

Understanding the mechanisms of disruption of maternal immunology tolerance to fetus, causes of transition of physiologic inflammatory reaction to systemic and excessive inflammatory response (as in PE), accompanied by endothelial activation/dysfunction, and revelation of the contribution of eGC damage to preeclampsia development may be subject of new discoveries in the disease pathogenesis.

3. The development of systemic inflammatory response in pregnancy

There is much experimental evidence of a so-called “physiological”, controlled SIR during pregnancy. Similarly to the classic inflammatory response, physiological inflammatory response during pregnancy is a reaction to local damage (matrix remodeling, associated with implantation, placentation and angiogenesis in placenta) [92, 93] and foreign invaders (cells, microparticles and soluble factors of placental origin) [94, 95]. Humoral factors, cellular debris and subcellular particles of trophoblast are considered to be the triggers of SIR, but they can also play a role of adjuvants [95, 96]. Cells-effectors of the maternal innate immunity detect fetal products as pathogen/danger images, implementing cell and molecular protection mechanisms against allogeneic material [97]. The gene products inherited from the father can be

regarded as exogenous factors, while endogenous factors are gene products, resulting from trauma, ischemia, necrosis or oxidative stress [97]. Also there are some reports on generation of various new antigens due to inflammatory response; they are variations of the "modified own"; of the neoantigens formed as a result of the post-translational proteins modification [98]; and of antigens, mobilized to membrane from cytoplasm and the inner cell compartments interacting with membrane proteins or phospholipids, and acting as images of danger [99]. The enhanced pro-inflammatory background in normal pregnancy is evidenced by an increase of the level of the soluble cell adhesion molecules (sCAM) in blood, indicating the activation of leukocytes (increase of sE-selectin, sVCAM-1, sICAM-1 levels) and endothelial cells [100, 101].

3.1. The glycan-mediated processes in inflammation

Central event of the inflammatory response is the contact between leukocytes and endothelium, with subsequent migration of immune cells to the inflammatory lesion. At early stages of inflammatory response endothelial selectins (E-selectin and P-selectin) and lymphocytic L-selectin form reversible bonds with carbohydrate counter-receptors on the partner cell, thus providing tethering and the leukocyte rolling along the vascular wall.

The counter-receptors for selectins are typically heavily glycosylated molecules, many of which bear terminal SLe^x motifs (Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc) [102]. P- and L-selectin, but not E-selectin, bind to some forms of heparin/HS. However, each of the selectins binds with higher affinity to its specific macromolecular ligands. Many of the known ligands are mucins containing sialylated fucosylated O-glycans. The major ligand for P-selectin, named P-selectin glycoprotein ligand-1 (PSGL-1), has sulfated tyrosine residues adjacent to a core-2 based O-glycan expressing SLe^x. Also, PSGL-1 is one of the physiological ligands for E-selectin, but E-selectin can also interact with several other glycoproteins that express the SLe^x motif on either N- or O-glycans, including the E-selectin ligand-1, CD44, L-selectin (in humans), and possibly long-chain GSLs expressing the SLe^x [68, 103]. Ligands for L-selectin that occur within specialized endothelia termed high endothelial venules (HEV; HEV are specialized post-capillary venous swellings characterized by plump endothelial cells as opposed to the usual thinner endothelial cells found in regular venules. HEVs enable lymphocytes circulating in the blood to directly enter a lymph node by crossing through the HEV) contain 6-sulfo-SLe^x motif on mucin-type O-glycans and on N-glycans [104]. The ligands for E- and P-selectin are expressed on circulating leukocytes whereas L-selectin binds to ligands on both leukocytes and the endothelium [89].

At the firm adhesion stage, following the leukocyte capture stage and rolling, N-linked glycans on ICAM-1 regulate binding to its integrin ligands—macrophage-1 antigen (Mac-1) and LFA-1. Moreover, it was found that Mac-1 binds with higher avidity to molecules of ICAM-1 with smaller N-linked oligosaccharide chains, since the binding with the ligand increased after the use of α-mannosidase inhibitor deoxymannojirimicin (DMJ). In contrast, LFA-1 binds with higher affinity to glycoforms of ICAM-1, which has a more complex carbohydrate chain [89]. Also, there is experimental evidence that high-mannose ICAM-1 can function in leukocyte firm-adhesion [105]. It is speculated that some N-glycan-binding sites on ICAM-1 may be

pro-adhesive, whereas the neighboring sites may be anti-adhesive, underscoring the potential breadth of how ICAM-1 function may be regulated by N-glycosylation [106]. On the stage of firm adhesion an important aspect of inflammatory response is exposure of the active epitope of integrins, provided by chemokines, which are present on the endothelial cell surface, and are bound to HS. Glycosylation of chemokine receptors also contributes to the adequate dynamics of the inflammatory reaction, thus increasing the binding affinity of the chemokine to the receptor and protecting the latter from proteolytic cleavage (reviewed in [18, 89]).

Key molecules mediating leukocyte transmigration: PECAM-1, JAM-1, ICAM-2 and VE-cadherin, are highly-glycosylated. However, carbohydrates part in leukocyte transmigration is still not clear. The recent studies show that N-glycosylation of JAM-A is required for the protein's ability to reinforce barrier function [22]; sialic acid-containing glycan of PECAM-1 reinforces dynamic endothelial cell-cell interactions by stabilizing the PECAM-1 homophilic binding interface [52]; glycosylation status of ICAM-2 (hypo- or non-glycosylated variants) significantly affects the function of this protein in cell motility assays [107]; in pro-inflammatory conditions, modification of VE-cadherin glycans is observed [55]. This obviously requires further investigations. Molecules that mediate intercellular interactions during inflammation are presented in **Table 3**.

Many studies demonstrate modification of endothelial glycome (glycome is the entire complement of sugars, whether free or present in more complex molecules, of an organism) under inflammatory response. Modeling of inflammatory response *in vitro* on endothelial cell lines showed that an enhanced α2,6-sialylation was observed after TNF stimulation [108]. Pro-inflammatory stimuli increase hypoglycosylated (namely, high-mannose/hybrid) N-glycans on the cell surface as determined by lectin histochemistry, and cause an increase in genes encoding for fucosylation and sialylation (confirmed at specific staining with relevant lectins [18]; this correlates with increased monocyte adhesion [18]. Glycosylation of the endothelium has been proposed to act as a "zip code" for directing leukocyte subtype-specific recruitment in different vascular beds in response to specific stimuli [89].

3.2. The glycobiology of immunoregulation

The carbohydrate-protein interactions not only mediate the initial stages of inflammation, but also promote many cellular contacts, which regulate innate and adaptive immune response. The main carbohydrate binding proteins are endogenous lectins [109], widely present on the immune system cells and expressed both in membrane-linked and in soluble forms. Three main classes of endogenous lectins include:

A. C-type lectins, which, depending of specificity, are:

- Specific to mannose (Man-) and/or fucose (Fuc-) terminated glycans;
- Specific to galactose (Gal-) or N-acetylgalactosamine (GalNAc-)/N-acetylglucosamine (GlcNAc-)

Lectins of C-type are present on macrophages, dendritic cells, natural killer cells, leukocytes. They act as pattern-recognition receptors and fulfill signaling and adhesion functions [110]. Glycoconjugates: bacterial lipooligosaccharides, peptidoglycans, and molecules emerged

Cell adhesion molecules (proteins)	Counter-receptors (carbohydrates)	Comments
L-selectin	**1.** MadCAM-1 **2.** CD34 **3.** Sgp200 **4.** GlyCAM-1 **5.** Endoglycan **6.** Endomucin **7.** PCLP **8.** PSGL-1 **9.** 6-sulfo-SLe^x determinant is associated with the MECA-79 epitope	Binding L-selectin with: • peripheral node addressins (no. 1, 2, 3, 4, 5, 6, 7) mediates lymphocyte recirculation (homing); • SLe^x-containing (no. 8) and sulfated glycans (no. 9) mediates leukocyte capture and rolling
P-selectin	**1.** PSGL-1 (major counter-receptor) **2.** heparin/heparin sulfate (binds weakly) **3.** some glycoproteins (mucins containing highly clustered glycans) that bear the SLe^x determinant	Mediates: • leukocyte recruitment in both acute and chronic inflammation; • leukocyte capture and rolling
E-selectin	**1.** PSGL-1 **2.** ESL-1 **3.** CD44 **4.** L-selectin (in humans)	glycoproteins that express the SLe^x antigen on either N- or O-glycans and possibly long-chain glycosphingolipids expressing the SLe^x antigen; Mediate: • recruit leukocytes recruitment to sites of inflammation; • leukocyte capture and rolling
ICAM-1	**1.** LFA-1 (αLβ2-integrin) **2.** Mac-1 (αMβ2-integrin)	Mediates the stage of firm adhesion of leukocytes to endothelium
VCAM-1	• VLA-4 (α4β1-integrin)	Mediates the stage of firm leucocytes adhesion of leukocytes to endothelium

MadCAM-1, mucosal addressin cell adhesion molecule-1; GlyCAM-1, glycosylation-dependent cell adhesion molecule-1; PCLP, podocalyxin-like protein; SLe^x sialyl-Lewis X; PSGL-1, P-selectin glycoprotein ligand 1; ESL-1, E-selectin ligand-1; LFA-1, lymphocyte function-associated antigen-1; Mac-1, macrophage-1 antigen; VLA-4, very late antigen-4.

Table 3. Molecules, mediating carbohydrate-protein interactions in inflammation site [80, 91, 92].

as a result of tissue damage: HA fragments or glycosaminoglycans of the extracellular cell matrix (ECM) and eGC [111], may act as pathogen/danger images for these lectins. The best known molecules related to C-type lectins are: selectins and myeloid range receptors (mannose-binding receptors DEC-205 and mannose receptor CD206); dectin-1 and dectin-2, DC-SIGN (CD209), and langerin (CD207) [112].

B. **Galectins** are a family of 15 evolutionary conserved carbohydrate-binding proteins [89, 113], belonging to the glycoproteins and glycolipids of cell surface and ECM [114] and specifically

binding mainly to N-acetyllactosamine. The main ligands are Galβ1-3GlcNAc- or Galβ1-4GlcNAc- [115]. Galectins are involved in many cell activities: cell cycle regulation, migration, cell signals transmission, effectory functions, apoptosis, immunoregulation [116]. Galectins may regulate inflammatory reaction both positively (Gal-3, Gal-8, Gal-9) and negatively (Gal-1). The endothelium may be a source of Gal-1, which then targets the neutrophils to inhibit cell recruitment, and Gal-3, Gal-8, Gal-9 promote neutrophil and eosinophil adhesion [89].

C. Siglecs are a family of 17 known lectins, which specifically bind the glycans structures with terminal sialic acid [117]. Sialyl Tn (Neu5Acα2,6GalNAcα-) is a common ligand for all members of this family. Glycan 6′ sulfated SLe^x is a ligand for Siglec-8, and is important for selectin-dependent cell adhesion [118]. The majority of this family members are inhibitory receptors as they bear an immunoreceptor tyrosine-based inhibition motif (ITIM) in their structure, and they are mainly expressed on immune cells [119]. Siglecs participate in regulation/restriction of an excessive activation response to inflammatory reaction, initiated via recognition of pathogen associated molecular patterns, and damage-associated molecular patterns, with following phagocytosis of cells, bearing these patterns [120, 121]. Siglecs regulate cell proliferation, differentiation, apoptosis, adhesion, cytokines synthesis and negative regulation of B-lymphocyte signaling [122].

Some endogenous lectins are capable, like autoantibodies, to interact with the body's unchanged antigens (glycans), so-called own self-images (SAMPs-self-associated molecular patterns) [111]. Molecular patterns, containing sialic acid and heparin/HS are supposed to act as self-images [111]. Also it is thought that interaction of lectins, recognizing SAMPs, (mainly siglecs), with ligands, inhibits the immune response to foreign/damaging effects [111, 120].

It is known that presence of terminal sialic acid is very important: this substance provides the overall negative charge of cell surface, glycoconjugates conformation stabilization, production of glycoconjugates, and cells protection from recognition and degradation. Sialylation protective properties manifest not only with sialylated structures interaction with inhibited receptors, but also with masking of sugar residues which are the antigen determinants [123, 124]. For example, at desialylation, the unmasked residues of Galβ-, GalNAc-, and mannose, interacting with lectins from galectins family and C-type lectins [120]; these interactions are important for metastasis and SIR development.

Therefore, inflammatory response regulation is implemented under direct involvement of the glycan binding proteins (endogenous lectins) and glycans; composition and structure of these vary significantly under physiological and pathophysiological conditions, providing evidence of the eGC modification at inflammation, and of formation of the carbohydrate "zip code", which acts as navigator for immune cells. Inflammatory reactions in pregnancy are initiated by pathogenic and danger images, which are formed at the fetal-mother cell contact; this activates innate and adaptive immunity. SIR may be enhanced or restricted through mechanisms based on carbohydrate-protein interaction [125–127]. Excessive SIR developing in pathologic pregnancy is characterized by compensatory reactions and development of various dysfunctions, resulting in organic or multi-organic failure [128].

http://dx.doi.org/10.5772/intechopen.75043

4. Endothelial activation and endothelial dysfunction

As a rule, in the studies dedicated to determination of endothelium role in different pathologies, the authors use terms "endothelial activation" and "endothelial dysfunction" [129]. Activation should be distinguished from activity because in its resting state, endothelium is a metabolically active organ, which produces vasodilatory substances and bears anticoagulative and antiadhesive phenotype. Activation of endothelium under various pathophysiologic processes leads to alterations of its phenotype and function. These events may be reversible, but also may cause multiorgan failure.

There are two stages in endothelial activation: endothelial stimulation (early events) and endothelial activation (later events). The latter can be subdivided in endothelial activation of types I and II, respectively [130, 131]. Endothelial activation of type I follows the stimulation stage and manifests with shedding of the adhesion molecules and molecules with antithrombotic properties, such as P-selectin, thrombin, heparin, antithrombin III and thrombomodulin, from the surface of the endothelial cells. In the same time, the endothelial cells of the venules and small veins decrease in volume, and the contacts between the cells become distorted, resulting in hemorrhages, edema, and increase of vessels permeability [131]. Endothelial activation of type II is a slightly delayed process, which depends on gene transcription activation and protein synthesis *de novo*. As a result, the genes coding for the adhesion molecules, chemokines and procoagulative factors: E-selectin, vWF, IL-8, thrombocytes activating factor [132], are activated. Also, the secretion of NO and prostacyclin increases. Morphologic changes show protrusion of the endothelial cells into the vessel lumen, cell hypertrophy and an increase of cell permeability. The result of this stage is leukocyte contact with activated endothelium through lectin-carbohydrate interactions, extravasation, transendothelial migration, and, possibly, leucocyte binding with Fc-receptors (FcR) of endothelial cells with immune complexes disposition [131]. Alterations of phenotype, accompanying endothelial cells activation, manifest also with the change of the carbohydrate composition of the molecules forming the eGC.

Therefore, endothelial activation implies an alteration of the endothelial cells phenotype under the activation factors (cytokines, endotoxins, etc.) impact, inducing shedding and modification of the vasculoprotective surface layer associated with the membrane, and expression of the activation antigens. This correlates with pro-adhesive, antigen-presenting and procoagulative properties of the endothelial cells. Activation reflects an ability of endothelial cells to perform new functions, but this status does not presume a cell damage or their uncontrolled division. Endothelium activation is a reverse process with a possibility to return to a state of active reposing cells [131].

Endothelium dysfunction, on the other hand, is a stage following the endothelium activation and manifesting with cell functional activity change; it leads to loss of the ability of endothelium to perform its function, and to a disbalance of factors, which provide homeostasis and a normal course of all processes, mediated by endothelium [8, 129, 131]. Endothelial dysfunction is a consequence of chronic, permanent endothelial activation and may lead to non-reversible damage of the endothelial cells, their apoptosis and necrosis.

5. Preeclampsia as a manifestation of excessive systemic inflammatory response, accompanied by endothelial activation/ dysfunction

PE is a multisystemic pathologic condition, manifesting after the 20th week of pregnancy. PE clinical signs are: an increase of systolic blood pressure (SBP) above 140 mm Hg, diastolic blood pressure (DBP) above 90 mm Hg for the first time noted during pregnancy; proteinuria (≥0.3 g/L) in daily urine, edema, manifestation of multiorganic/polysystemic dysfunction/ insufficiency [133]. Severe PE is accompanied by acute renal failure, eclampsia, pulmonary edema, HELLP (hemolysis, elevated liver enzymes, and low platelet count)-syndrome [3].

Etiology of PE is not clear; genetic, immunological and microenvironment may play a role [134–138]. Currently two phenotypic variations of PE are distinguished: early manifestation of the symptoms (before the 34th week of gestation) and later manifestation (after the 34th week of gestation) [139]. Pathophysiological mechanisms of PE development are distinguished accordingly [140]. The first—"fetal" pathway—is characterized by inadequate or microcellular invasion of trophoblast cells into the uterine spiral arteries and lack or incompleteness of the phase of substitution of placental smooth muscle elastic fibers with fibrinoid [140, 141]. In this mechanism, physiological remodeling and transformation of spiral arteries is lacking, and this affects the uterine-placentary blood flow quality [142–144]. Fetal mechanism of PE development presents with severe disease course and frequent complications in the neonate. The second pathway is "maternal", where the deficiency of uterine-placental blood flow appears as a result of spiral arteries damage due to certain maternal diseases, especially thrombophilias (genetic or acquired). In this case, the study of placental morphology testifies adequate gestational reorganization of spiral arteries. Maternal pathway usually implies later manifestation and a milder course. Some also distinguish the third (or "mixed") pathway, where the arteries are both affected and poorly reorganized [145, 146].

Disrupted trophoblast invasion initiates ischemic and hypoxic damage of placental cells and tissues, leading to increase of cell debris and microparticles of fetal origin contents in the mother's blood. These processes result in the mother's immune cells activation and inflammatory cytokines synthesis induction [147], leading to the development of generalized endothelial activation/dysfunction with development of multiorganic insufficiency [148] (**Figure 1**). Trophoblast debris was also found in the mother's is blood in a normal pregnancy and it was primarily apoptotic. Particles of trophoblast debris range from polynuclear aggregates of the syncytium cells to subcellular micro and nanoparticles. *In vitro* co-culturing of trophoblast debris, obtained from women with normal pregnancy, with macrophages and endothelial cells leads to tolerogenic M2-phenotype of macrophages [149, 150]. Trophoblast debris becomes more necrotic when *in vitro* system is supplemented with antiphospholipid antibodies or IL-6. Phagocytosis of the necrotic debris by the endothelial cells is accompanied by their activation [151]. Activation of endothelial cells is also caused by the addition of the trophoblast debris isolated from patients with preeclampsia to the culture of the endothelial cells [152].

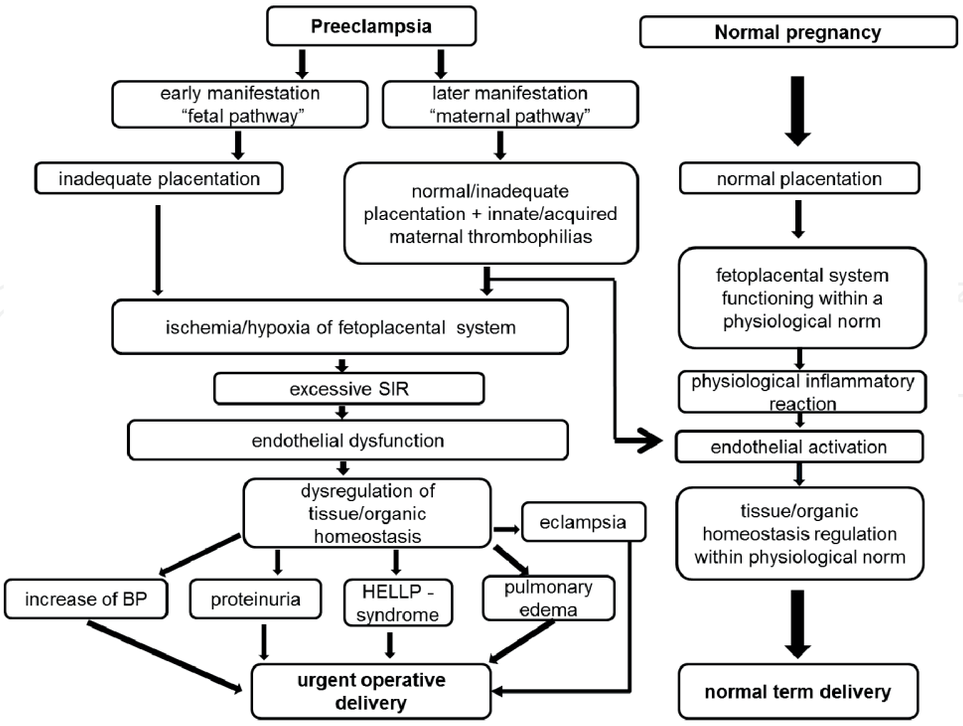

Figure 1. Modern concept of the pathogenesis of preeclampsia (PE). Two phenotypic PE variations (early onset-PE and late onset-PE) exhibit different pathophysiology and clinical outcome. Clinical manifestations of endothelial activation/dysfunction are expressed in various degree and in both forms. SIR, systemic inflammatory response; BP, blood pressure; HELLP, hemolysis, elevated liver enzymes, and low platelet count.

5.1. Endothelium activation markers in preeclampsia

Numerous studies have shown that in PE, manifestations of excessive SIR are observed due to the loss of control over the balance of production of pro/anti-inflammatory cytokines. This leads to an increase in the synthesis and expression of key molecules that mediate intercellular contacts between leukocytes and endothelium [147, 153, 154]. In this context, it has been shown that in PE, the plasma levels of sE-selectin, sVCAM-1 and sICAM-1 were significantly elevated [100, 155–157], and that cultivation of endothelial cells with the blood serum of PE women significantly increased the expression of ICAM-1 by the endothelial cells [158].

It was found that the expression of E-selectin and P-selectin in the endothelial cell culture was significantly higher after administration of trophoblast cells from the PE patients, than after cultivation of endothelial cells with trophoblast cells isolated from placental tissue of healthy women [159]. We have shown in a prospective longitudinal study that in patients with severe PE, the levels of sE-selectin, sVCAM-1 and sICAM-1 were increased from the 8th week of pregnancy until the appearance of clinical symptoms of the disease [160]. In a similar design study, it was shown that joint determination of sICAM-1 and sVCAM-1 levels measured in peripheral blood within 22–29 weeks of gestation, was of high predictive value and capable to detect up to 55% of women with a pathologic pregnancy [161]. The increased levels of sICAM-1 and sVCAM-1 in blood during PE significantly correlated with the signs of the acute phase of inflammation and PE: hypertension, proteinuria, increase of hepatic enzymes levels

[162]. Also it was noted that high levels of sVCAM-1 and sE-selectin in women with PE could result in adverse perinatal outcome and endothelial dysfunction in fetus, as confirmed by negative correlation between sVCAM-1 and endogenous NO synthesis by HUVECs, isolated from the umbilical cord after birth [163].

5.2. Alteration of endothelial glycocalyx in preeclampsia

The signs of endothelial activation are the expression of activation markers by endothelial cells and increased plasma concentrations of the soluble forms of CAMs and of the factors, regulating angiogenesis and blood clotting. However, the main feature of the evolving endothelial activation is alteration, damage and shedding of the eGC and an increase of its components concentration in blood. Currently, there are limited studies of this phenomena in PE, but available reports show significant alteration of eGC composition in the placental structures in PE [164]. The most prominent alteration of the eGC composition was found in the placentas of women with severe PE. Alterations take place also in the eGC capillaries of terminal placental villi: the content of glycans with terminal β-galactosyl and α-mannosyl residues increase, while the content of α2,3-linked sialic acids decrease in the glycome in severe PE [165]. These alterations are supposed to point to the exposure of glycans bearing the "danger signals" and being the counter-receptors for endogenous lectins; interaction with these activate maternal immune system [166, 167] (REF). Such studies, performed by immunohistochemistry of placenta after childbirth and using the lectins panel or monoclonal antibodies to carbohydrates antigens, give an idea of alterations of the placental glycome and its separate structures, including capillary endothelium, and provide evidence obtained by direct eGC visualization [165, 168]. Since direct visualization of the eGC is impossible in clinical trials where no surgical tissue sampling is implied, in these cases, an indirect assessment of the content of the degradation products of eGC is used.

Indirect methods have significant limitations, but they are the only possibility to evaluate the eGC *in vivo*. Indirect assessment of the eGC by ELISA show that in PE, the plasma content of the structural proteoglycans (endocan-1, syndecan-1, decorin and HA) and the GAGs of eGC increase [169–171]. Serum endocan concentrations were significantly elevated in women with PE versus normotensive controls, and concentrations seem to be associated with the severity of the disease [172]. Median maternal plasma endocan concentrations were higher in PE patients and lower in acute pyelonephritis with bacteremia than in uncomplicated pregnancy. No significant difference was observed in the median plasma endocan concentration between other obstetrical syndromes and uncomplicated pregnancies [173]. It is suggested that in PE, the maternal endothelium is a source of GAGs in blood, and intensive eGC shedding thus indicates a manifestation of endothelial dysfunction [169–174]. Also, patients with PE show GAGs excretion in urine; this is thought to be linked with the eGC proteoglycans alterations and with the glomerular basement membrane changes, and associated with proteinuria [175]. *In vitro* and *in vivo* experimental studies, using cell and animal models is another opportunity of indirect eGC evaluation. This approach was used to study CKD [74, 75], cardio-vascular and inflammatory diseases [13, 176], cancer [13, 176, 177]—the conditions manifestating with hypertension, proteinuria, edema, SIR, thrombosis. The results of such studies provide some

keys to PE, which is less studied, but exhibits similar clinical signs. Experimental models allow to evaluate not only the degree of the eGC damage by various factors (SIR being the most significant), but also the molecular changes of the eGC composition. This moment is a crucial point because SIR is not a specific process; it accompanies almost any pathology and promotes the generation of neoantigens, acting as an adaptive response trigger and provoking autoimmune reactions.

6. Conclusion

Endothelial dysfunction represents the central link in the pathogenesis of various diseases and complications, and is a subject of intensive research. On the background of the progress in understanding the mechanisms of development, diagnosis and treatment of endothelial dysfunction, many studies in the recent years have been focused on the eGC as an early indicator of endothelial injury and a potential marker of vascular injury.

Alterations of the phenotype of endothelial cells, secretion and release of various activation markers into the bloodstream and dysfunction of the endothelium are directly related to the damage of eGC. This damage is the initiating factor and the initial stage in the development of endothelial activation/dysfunction, but this stage has for a long time been obscure due to the difficulties of eGC visualization and diagnosis.

By now, the main criteria for eGC damage assessment have been defined. In addition to the appearance of eGC components in the blood, the degree of manifestation of the SIR is also an important criterium of the damage, since endothelial inflammation and dysfunction are inseparably related processes. In this regard, the molecular mechanism of the inflammatory reaction is based on the ligand-receptor, carbohydrate-protein interaction of the immune cells and endothelium, and alteration of glycome/glycocalyx is a crucial factor in the development of inflammation and endothelial dysfunction. Therefore, the pathogenesis of endothelial activation/dysfunction should be envisioned from the point of damage of the intravascular compartment—the eGC, which regulates the functions of the endothelium.

Expanding research of the eGC role in the development of endothelial dysfunction may be a subject of new discoveries in the pathogenesis of a large group of diseases, including pregnancy pathology and PE, especially since PE is a classic example of the immune system hyperactivation, manifestation of SIR and development of endothelial dysfunction. Undoubtedly, future studies of the eGC will evoke an absolutely new insight in the development and progression of endothelial dysfunction.

Acknowledgements

We thank Alexey Komarovsky and Natalia Baranova for their help in the manuscript preparation.

Conflict of interest

The authors report no conflicts of interest.

Notes/thanks/other declarations

The study has been carried out within the State Contract (№ 116082210002) "Investigation of diagnostic and prognostic value of molecular-genetic, immunologic, epigenetic factors in pre-eclampsia."

Author details

Marina M. Ziganshina[1]*, Ekaterina L. Yarotskaya[2], Nicolai V. Bovin[3] and Gennady T. Sukhikh[1]

*Address all correspondence to: mmz@mail.ru

1 Laboratory of Clinical Immunology, Federal State Budget Institution National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Healthcare of Russian Federation, Moscow, Russian Federation

2 Department of International Cooperation, Federal State Budget Institution National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I. Kulakov of the Ministry of Healthcare of Russian Federation, Moscow, Russian Federation

3 Laboratory of Carbohydrates of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Russian Federation

References

[1] Valdiviezo C, Garovic VD, Ouyang P. Preeclampsia and hypertensive disease in pregnancy: Their contributions to cardiovascular risk. Clinical Cardiology. 2012;**35**:160-165. DOI: 10.1002/clc.21965

[2] Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P. Diagnosis, evaluation, and management of the hypertensive disorders of pregnancy: Executive summary. Journal of Obstetrics and Gynaecology Canada. 2014;**36**:575-576. DOI: 10.1016/S1701-2163(15)30533-8

[3] Ramma W, Ahmed A. Is inflammation the cause of pre-eclampsia? Biochemical Society Transactions. 2011;**39**(6):1619-1627. DOI: 10.1042/BST20110672

[4] Kim Y, Romero R, Oh S, Kim C, Kilburn B, Armant D, Nien J, Gomez R, Mazor M, Saito S, Abrahams V, Mor G. Toll-like receptor 4: A potential link between "danger signals," the innate immune system, and preeclampsia? American Journal of Obstetrics and Gynecology. 2005;**193**(3 Pt 2):921-927. DOI: 10.1016/j.ajog.2005.07.076

[5] Bernardi F, Felisberto F, Vuolo F, Petronilho F, Souza D, Luciano T, de Souza C, Ritter C, Dal-Pizzol F. Oxidative damage, inflammation, and Toll-like receptor 4 pathway are increased in preeclamptic patients: A case-control study. Oxidative Medicine and Cellular Longevity. 2012;**2012**:636419. DOI: 10.1155/2012/636419

[6] Scott DW, Vallejo MO, Patel RP. Heterogenic endothelial responses to inflammation: Role for differential N-glycosylation and vascular bed of origin. Journal of the American Heart Association. 2013;**2**(4):e000263. DOI: 10.1161/JAHA.113.000263

[7] Fu BM, Tarbell JM. Mechano-sensing and transduction by endothelial surface glycocalyx: Composition, structure, and function. Wiley Interdisciplinary Reviews. Systems Biology and Medicine. 2013;**5**(3):381-390. DOI: 10.1002/wsbm.1211

[8] Aird WC. Endothelium in health and disease. Pharmacological Reports. 2008;**60**(1):139-143

[9] Pries AR, Kuebler WM. Normal endothelium. Handbook of Experimental Pharmacology. 2006;**176**(Pt 1):1-40

[10] Galley HF, Webster NR. Physiology of the endothelium. British Journal of Anaesthesia. 2004;**93**(1):105-113. DOI: 10.1093/bja/aeh163

[11] Schött U, Solomon C, Fries D, Bentzer P. The endothelial glycocalyx and its disruption, protection and regeneration: A narrative review. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine. 2016;**24**:48. DOI: 10.1186/s13049-016-0239-y

[12] Becker BF, Chappell D, Bruegger D, Annecke T, Jacob M. Therapeutic strategies targeting the endothelial glycocalyx: Acute deficits, but great potential. Cardiovascular Research. 2010;**87**(2):300-310. DOI: 10.1093/cvr/cvq137

[13] Tarbell JM, Cancel LM. The glycocalyx and its significance in human medicine. Journal of Internal Medicine. 2016;**280**(1):97-113. DOI: 10.1111/joim.12465

[14] Ziganshina MM, Pavlovich SV, Bovin NV, Sukhikh GT. Hyaluronic acid in vascular and immune homeostasis during normal pregnancy and preeclampsia. Acta Naturae. 2016; **8**(3):59-71

[15] Reitsma S, Slaaf DW, Vink H, van Zandvoort MA, oude Egbrink MG. The endothelial glycocalyx: Composition, functions, and visualization. Pflügers Archiv 2007;**454**(3):345-359. DOI: 10.1007/s00424-007-0212-8

[16] Maksimenko AV. Translational research into vascular wall function: Regulatory effects of systemic and specific factors. Journal of Translational Science. 2017;**3**(2):1-10. DOI: 10.15761/JTS.1000180

[17] Jiménez D, Roda-Navarro P, Springer TA, Casasnovas JM. Contribution of N-linked glycans to the conformation and function of intercellular adhesion molecules (ICAMs). The Journal of Biological Chemistry. 2005;**280**(7):5854-5861. DOI: 10.1074/jbc.M412104200

[18] Scott DW, Patel RP. Endothelial heterogeneity and adhesion molecules N-glycosylation: Implications in leukocyte trafficking in inflammation. Glycobiology. 2013;**23**(6):622-633. DOI: 10.1093/glycob/cwt014

[19] Rüegg C, Mariotti A. Vascular integrins: Pleiotropic adhesion and signaling molecules in vascular homeostasis and angiogenesis. Cellular and Molecular Life Sciences. 2003;**60**(6):1135-1157. DOI: 10.1007/s00018-003-2297-3

[20] Hang Q, Isaji T, Hou S, Zhou Y, Fukuda T, Gu J. N-glycosylation of integrin α5 acts as a switch for EGFR-mediated complex formation of integrin α5β1 to α6β4. Scientific Reports. 2016;**6**:33507. DOI: 10.1038/srep33507

[21] Geyer H, Geyer R, Odenthal-Schnittler M, Schnittler HJ. Characterization of human vascular endothelial cadherin glycans. Glycobiology. 1999;**9**(9):915-925

[22] Scott DW, Tolbert CE, Graham DM, Wittchen E, Bear JE, Burridge K. N-glycosylation controls the function of junctional adhesion molecule-A. Molecular Biology of the Cell. 2015;**26**(18):3205-3214. DOI: 10.1091/mbc.E14-12-1604

[23] https://www.omim.org/entry/606870#7

[24] Arrate MP, Rodriguez JM, Tran TM, Brock TA, Cunningham SA. Cloning of human junctional adhesion molecule 3 (JAM3) and its identification as the JAM2counter-receptor. The Journal of Biological Chemistry. 2001;**276**(49):45826-45832. DOI: 10.1074/jbc.M105972200

[25] Casasnovas JM, Springer TA, Liu JH, Harrison SC, Wang JH. Crystal structure of ICAM-2 reveals a distinctive integrin recognition surface. Nature. 1997;**387**(6630):312-315. DOI: 10.1038/387312a0

[26] Imai Y, Lasky LA, Rosen SD. Sulphation requirement for GlyCAM-1, an endothelial ligand for L-selectin. Nature. 1993;**361**(6412):555-557. DOI: 10.1038/361555a0

[27] Hemmerich S, Leffler H, Rosen SD. Structure of the O-glycans in GlyCAM-1, an endothelial-derived ligand for L-selectin. Journal of Biological Chemistry. 1995;**270**(20):12035-12047

[28] Mir GH, Helin J, Skarp KP, Cummings RD, Mäkitie A, Renkonen R, Leppänen A. Glycoforms of human endothelial CD34 that bind L-selectin carry sulfated sialyl Lewis x capped O- and N-glycans. Blood. 2009;**114**(3):733-741. DOI: 10.1182/blood-2009-03-210237

[29] Wu W, Pasternack L, Hua Huang D, Koeller KM, Lin C-C, Seitz O, Wong C-H. Structural study on O-glycopeptides: Glycosylation-induced conformational changes of O-GlcNAc, O-LacNAc, O-Sialyl-LacNAc, and O-Sialyl-Lewis-X peptides of the Mucin domain of MAdCAM-1. Journal of the American Chemical Society. 1999;**121**(11):2409-2417. DOI: 10.1021/ja983474v

[30] Solecka BA, Weise C, Laffan MA, Kannicht C. Site-specific analysis of von Willebrand factor O-glycosylation. Journal of Thrombosis and Haemostasis. 2016;**14**(4):733-746. DOI: 10.1111/jth.13260

[31] Canis K, McKinnon TA, Nowak A, Haslam SM, Panico M, Morris HR, Laffan MA, Dell A. Mapping the N-glycome of human von Willebrand factor. The Biochemical Journal. 2012;**447**(2):217-228. DOI: 10.1042/BJ20120810

[32] Koyama T, Parkinson JF, Sié P, Bang NU, Müller-Berghaus G, Preissner KT. Different glycoforms of human thrombomodulin. Their glycosaminoglycan-dependent modulatory effects on thrombin inactivation by heparin cofactor II and antithrombin II. European Journal of Biochemistry. 1991;**198**(3):563-570

[33] Edano T, Inoue K, Yoshizaki H, Yamamoto S, Komine N, Tabunoki H, Sawada H, Koshi T, Murakami A, Wada Y, Ohkuchi M. Increased anticoagulant activity of recombinant thrombomodulin modified with glycosaminoglycan. Biological & Pharmaceutical Bulletin. 1998;**21**(4):375-381

[34] Edano T, Kumai N, Mizoguchi T, Ohkuchi M. The glycosylation sites and structural characteristics of oligosaccharides on recombinant human thrombomodulin. The International Journal of Biochemistry & Cell Biology. 1998;**30**(1):77-88

[35] Nadanaka S, Kitagawa H, Sugahara K. Demonstration of the immature glycosaminoglycan tetrasaccharide sequence GlcAb1-3Galb1-3Galb1-4Xyl on recombinant soluble human a-thrombomodulin. JBC. 1998;**273**(50):33728-33734

[36] Wakabayashi H, Natsuka S, Honda M, Naotsuka M, Ito Y, Kajihara J, Hase S. Structural analysis of the sugar chains of human urinary thrombomodulin. Journal of Biochemistry. 2001;**130**(4):543-552

[37] Ito T, Maruyama I. Thrombomodulin: Protectorate god of the vasculature in thrombosis and inflammation. Journal of Thrombosis and Haemostasis. 2011;**9**(Suppl. 1):168-173. DOI: 10.1111/j.1538-7836.2011.04319.x

[38] Martínez-Martínez I, Dichiara G, Gutiérrez-Gallego R, Navarro-Fernández J, Vicente V, Corral J. Increased N-glycosylation efficiency by generation of an aromatic sequon on N135 of antithrombin. PLoS One. 2014;**9**(12):e114454. DOI: 10.1371/journal.pone.0114454

[39] Kumar A, Bhandari A, Sarde SJ, Goswami C. Sequence, phylogenetic and variant analyses of antithrombin III. Biochemical and Biophysical Research Communications. 2013;**440**(4):714-724. DOI: 10.1016/j.bbrc.2013.09.134

[40] Böhme C, Nimtz M, Grabenhorst E, Conradt HS, Strathmann A, Ragg H. Tyrosine sulfation and N-glycosylation of human heparin cofactor II from plasma and recombinant Chinese hamster ovary cells and their effects on heparin binding. European Journal of Biochemistry. 2002;**269**(3):977-988

[41] Shriver Z, Capila I, Venkataraman G, Sasisekharan R. Heparin and heparin sulfate: Analyzing structure and microheterogeneity. Handbook of Experimental Pharmacology. 2012;**207**:159-176. DOI: 10.1007/978-3-642-23056-1_8

[42] Sugiura N, Shioiri T, Chiba M, Sato T, Narimatsu H, Kimata K, Watanabe H. Construction of a chondroitin sulfate library with defined structures and analysis of molecular interactions. The Journal of Biological Chemistry. 2012;**287**(52):43390-43400. DOI: 10.1074/jbc.M112.412676

[43] Akatsu C, Mizumoto S, Kaneiwa T, Maccarana M, Malmström A, Yamada S, Sugahara K. Dermatan sulfate epimerase 2 is the predominant isozyme in the formation of the chondroitin sulfate/dermatan sulfate hybrid structure in postnatal developing mouse brain. Glycobiology. 2011;**21**(5):565-574. DOI: 10.1093/glycob/cwq208

[44] Higashi K, Takeda K, Mukuno A, Okamoto Y, Masuko S, Linhardt RJ, Toida T. Identification of keratin sulfate disaccharide at C-3 position of glucuronate of chondroitin sulfate from *Mactra chinensis*. The Biochemical Journal. 2016;**473**(22):4145-4158. DOI: 10.1042/BCJ20160655

[45] Gubbiotti MA, Neill T, Iozzo RV. A current view of perlecan in physiology and pathology: A mosaic of functions. Matrix Biology. 2017;**57-58**:285-298. DOI: 10.1016/j.matbio.2016.09.003

[46] Sotoodehnejadnematalahi F, Burke B. Structure, function and regulation of versican: The most abundant type of proteoglycan in the extracellular matrix. Acta Medica Iranica. 2013;**51**(11):740-750

[47] Kenagy RD, Plaas AH, Wight TN. Versican degradation and vascular disease. Trends in Cardiovascular Medicine. 2006;**16**(6):209-215. DOI: 10.1016/j.tcm.2006.03.011

[48] Kali A, Shetty KS. Endocan: A novel circulating proteoglycan. Indian Journal of Pharmacology. 2014;**46**(6):579-583. DOI: 10.4103/0253-7613.144891

[49] Seidler DG, Dreier R. Decorin and its galactosaminoglycan chain: Extracellular regulator of cellular function? IUBMB Life. 2008;**60**(11):729-733. DOI: 10.1002/iub.115

[50] Nastase MV, Young MF, Schaefer L. Biglycan: A multivalent proteoglycan providing structure and signals. The Journal of Histochemistry and Cytochemistry. 2012;**60**(12):963-975. DOI: 10.1369/0022155412456380

[51] Newton JP, Hunter AP, Simmons DL, Buckley CD, Harvey DJ. CD31 (PECAM-1) exists as a dimer and is heavily N-glycosylated. Biochemical and Biophysical Research Communications. 1999;**261**(2):283-291. DOI: 10.1006/bbrc.1999.1018

[52] Lertkiatmongkol P, Paddock C, Newman DK, Zhu J, Thomas MJ, Newman PJ. The role of sialylated glycans in human platelet endothelial cell adhesion molecule 1 (PECAM-1)-mediated trans homophilic interactions and endothelial cell barrier function. The Journal of Biological Chemistry. 2016;**291**(50):26216-26225. DOI: 10.1074/jbc.M116.756502

[53] Påhlsson P, Strindhall J, Srinivas U, Lundblad A. Role of N-linked glycosylation in expression of E-selectin on human endothelial cells. European Journal of Immunology. 1995;**25**(9):2452-2459. DOI: 10.1002/eji.1830250907

[54] Yoshimura M, Ihara Y, Matsuzawa Y, Taniguchi N. Aberrant glycosylation of E-cadherin enhances cell-cell binding to suppress metastasis. The Journal of Biological Chemistry. 1996;**271**(23):13811-13815

[55] Sasaki N, Toyoda M. Glycoconjugates and related molecules in human vascular endothelial cells. International Journal of Vascular Medicine. 2013;**2013**:963596. DOI: 10.1155/2013/963596

[56] McCoy AJ, Pei XY, Skinner R, Abrahams JP, Carrell RW. Structure of beta-antithrombin and the effect of glycosylation on antithrombin's heparin affinity and activity. Journal of Molecular Biology. 2003;**326**(3):823-833

[57] Sperandio M, Gleissner CA, Ley K. Glycosylation in immune cell trafficking. Immunological Reviews. 2009;**230**(1):97-113. DOI: 10.1111/j.1600-065X.2009.00795

[58] Schnaar RL, Suzuki A, Stanley P. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 10 Glycosphingolipids

[59] Iwabuchi K. Involvement of glycosphingolipid-enriched lipid rafts in inflammatory responses. Frontiers in Bioscience. 2015;**20**:325-334

[60] Gillard BK, Jones MA, Marcus DM. Glycosphingolipids of human umbilical vein endothelial cells and smooth muscle cells. Archives of Biochemistry and Biophysics. 1987; **256**(2):435-445

[61] Nakayama H, Yoshizaki F, Prinetti A, Sonnino S, Mauri L, Takamori K, Ogawa H, Iwabuchi K. Lyn-coupled LacCer-enriched lipid rafts are required for CD11b/CD18-mediated neutrophil phagocytosis of nonopsonized microorganisms. Journal of Leukocyte Biology. 2008;**83**(3):728-741. DOI: 10.1189/jlb.0707478

[62] Iwabuchi K, Nagaoka I. Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils. Blood. 2002;**100**(4):1454-1464

[63] Iwabuchi K, Nakayama H, Oizumi A, Suga Y, Ogawa H, Takamori K. Role of ceramide from glycosphingolipids and its metabolites in immunological and inflammatory responses in humans. Mediators of Inflammation. 2015;**2015**:120748. DOI: 10.1155/2015/120748

[64] Zemunik T, Markotić A, Marusić A. Expression of neutral glycosphingolipids in cytokine-stimulated human endothelial cells. Biochemistry (Mosc). 2004;**69**(5):513-519

[65] van de Kar NC, Kooistra T, Vermeer M, Lesslauer W, Monnens LA, van Hinsbergh VW. Tumor necrosis factor alpha induces endothelial galactosyl transferase activity and verocytotoxin receptors. Role of specific tumor necrosis factor receptors and protein kinase C. Blood. 1995;**85**(3):734-743

[66] Schweppe CH, Bielaszewska M, Pohlentz G, Friedrich AW, Büntemeyer H, Schmidt MA, Kim KS, Peter-Katalinić J, Karch H, Müthing J. Glycosphingolipids in vascular endothelial cells: Relationship of heterogeneity in Gb3Cer/CD77 receptor expression with differential Shiga toxin 1 cytotoxicity. Glycoconjugate Journal. 2008;**25**(4):291-304. DOI: 10.1007/s10719-007-9091-7

[67] Müthing J, Duvar S, Heitmann D, Hanisch FG, Neumann U, Lochnit G, Geyer R, Peter-Katalinic J. Isolation and structural characterization of glycosphingolipids of in vitro propagated human umbilical vein endothelial cells. Glycobiology. 1999;**9**(5):459-468

[68] Cooling LW, Zhang DS, Koerner TAW. Lewis X and sialyl Lewis X glycosphingolipids. Trends in Glycoscience and Glycotechnology. 1997;**9**(46):191-209

[69] Zeng Y. Endothelial glycocalyx as a critical signalling platform integrating the extracellular haemodynamic forces and chemical signalling. Journal of Cellular and Molecular Medicine. 2017;**21**(8):1457-1462. DOI: 10.1111/jcmm.13081

[70] Weinbaum S, Zhang X, Han Y, Vink H, Cowin SC. Mechanotransduction and flow across the endothelial glycocalyx. Proceedings of the National Academy of Sciences. 2003;**100**(13):7988-7995. DOI: 10.1073/pnas.1332808100

[71] Secomb TW, Hsu R, Pries AR. Effect of the endothelial surface layer on transmission of fluid shear stress to endothelial cells. Biorheology. 2001;**38**(2-3):143-150

[72] Satchell S. The role of the glomerular endothelium in albumin handling. Nature Reviews. Nephrology. 2013;**9**(12):717-725. DOI: 10.1038/nrneph.2013.197

[73] Salmon AH, Satchell SC. Endothelial glycocalyx dysfunction in disease: Albuminuria and increased microvascular permeability. The Journal of Pathology. 2012;**226**(4):562-574. DOI: 10.1002/path.3964

[74] Salmon AH, Ferguson JK, Burford JL, Gevorgyan H, Nakano D, Harper SJ, Bates DO, Peti-Peterdi J. Loss of the endothelial glycocalyx links albuminuria and vascular dysfunction. Journal of the American Society of Nephrology. 2012;**23**(8):1339-1350. DOI: 10.1681/ASN.2012010017

[75] Padberg JS, Wiesinger A, di Marco GS, Reuter S, Grabner A, Kentrup D, Lukasz A, Oberleithner H, Pavenstädt H, Brand M, Kümpers P. Damage of the endothelial glycocalyx in chronic kidney disease. Atherosclerosis. 2014;**234**(2):335-343. DOI: 10.1016/j.atherosclerosis.2014.03.016

[76] Dane MJ, van den Berg BM, Avramut MC, Faas FG, van der Vlag J, Rops AL, Ravelli RB, Koster BJ, van Zonneveld AJ, Vink H, Rabelink TJ. Glomerular endothelial surface layer acts as a barrier against albumin filtration. The American Journal of Pathology. 2013;**182**(5):1532-1540. DOI: 10.1016/j.ajpath.2013.01.049

[77] Lennon FE, Singleton PA. Hyaluronan regulation of vascular integrity. American Journal of Cardiovascular Disease. 2011;**1**(3):200-213

[78] Curry FR, Adamson RH. Tonic regulation of vascular permeability. Acta Physiologica. 2013;**207**(4):628-649. DOI: 10.1111/apha.12076

[79] Lipowsky HH. The endothelial glycocalyx as a barrier to leukocyte adhesion and its mediation by extracellular proteases. Annals of Biomedical Engineering. 2012;**40**(4):840-848. DOI: 10.1007/s10439-011-0427-x

[80] Kolářová H, Ambrůzová B, Svihálková Šindlerová L, Klinke A, Kubala L. Modulation of endothelial glycocalyx structure under inflammatory conditions. Mediators of Inflammation. 2014;**2014**:694312. DOI: 10.1155/2014/694312

[81] Ushiyama A, Kataoka H, Iijima T. Glycocalyx and its involvement in clinical pathophysiologies. Journal of Intensive Care. 2016;**4**(1):59. DOI: 10.1186/s40560-016-0182-z

[82] McDonald KK, Cooper S, Danielzak L, Leask RL. Glycocalyx degradation induces a pro-inflammatory phenotype and increased leukocyte adhesion in cultured endothelial cells under flow. PLoS One. 2016;**11**(12):e0167576. DOI: 10.1371/journal.pone.0167576

[83] Henry CB, Duling BR. TNF-alpha increases entry of macromolecules into luminal endothelial cell glycocalyx. American Journal of Physiology. Heart and Circulatory Physiology. 2000;**279**(6):H2815-H2823

[84] Constantinescu AA, Vink H, Spaan JA. Elevated capillary tube hematocrit reflects degradation of endothelial cell glycocalyx by oxidized LDL. American Journal of Physiology. Heart and Circulatory Physiology. 2001;**280**(3):H1051-H1057

[85] Mulivor AW, Lipowsky HH. Inflammation- and ischemia-induced shedding of venular glycocalyx. American Journal of Physiology. Heart and Circulatory Physiology. 2004;**286**(5):H1672-H1680. DOI: 10.1152/ajpheart.00832.2003

[86] Lipowsky HH, Gao L, Lescanic A. Shedding of the endothelial glycocalyx in arterioles, capillaries, and venules and its effect on capillary hemodynamics during inflammation. American Journal of Physiology. Heart and Circulatory Physiology. 2011;**301**(6): H2235-H2245. DOI: 10.1152/ajpheart.00803.2011

[87] Rodrigues SF, Granger DN. Blood cells and endothelial barrier function. Tissue Barriers. 2015;**3**(1-2):e978720. DOI: 10.4161/21688370.2014.978720

[88] Termeer CC, Hennies J, Voith U, Ahrens T, Weiss JM, Prehm P, Simon JC. Oligosaccharides of hyaluronan are potent activators of dendritic cells. Journal of Immunology. 2000;**165**(4):1863-1870

[89] Wright RD, Cooper D. Glycobiology of leukocyte trafficking in inflammation. Glycobiology. 2014;**24**(12):1242-1251. DOI: 10.1093/glycob/cwu101

[90] Nieuwdorp M, Meuwese MC, Vink H, Hoekstra JB, Kastelein JJ, Stroes ES. The endothelial glycocalyx: A potential barrier between health and vascular disease. Current Opinion in Lipidology. 2005;**16**(5):507-511

[91] Vink H, Constantinescu AA, Spaan JA. Oxidized lipoproteins degrade the endothelial surface layer: Implications for platelet-endothelial cell adhesion. Circulation. 2000;**101**(13):1500-1502

[92] Romero R, Gotsch F, Pineles B, Kusanovic JP. Inflammation in pregnancy: Its roles in reproductive physiology, obstetrical complications, and fetal injury. Nutrition Reviews. 2007;**65**(12 Pt 2):S194-S202

[93] Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, Petraglia F. Inflammation and pregnancy. Reproductive Sciences. 2009;**16**(2):206-215. DOI: 10.1177/1933719108329095

[94] Jabbour HN, Sales KJ, Catalano RD, Norman JE. Inflammatory pathways in female reproductive health and disease. Reproduction. 2009;**138**(6):903-919. DOI: 10.1530/REP-09-0247

[95] Germain SJ, Sacks GP, Sooranna SR, Sargent IL, Redman CW. Systemic inflammatory priming in normal pregnancy and preeclampsia: The role of circulating syncytiotrophoblast microparticles. Journal of Immunology. 2007;**178**(9):5949-5956

[96] Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunology Today. 1999;**20**(3):114-118

[97] Redman CW, Sargent IL. Preeclampsia and the systemic inflammatory response. Seminars in Nephrology. 2004;**24**(6):565-570

[98] Eggleton P, Haigh R, Winyard PG. Consequence of neo-antigenicity of the 'altered self'. Rheumatology. 2008;**47**(5):567-571. DOI: 10.1093/rheumatology/ken014

[99] Keswani SC, Chauhan N. Antiphospholipid syndrome. Journal of the Royal Society of Medicine. 2002;**95**(7):336-342

[100] Lyall F, Greer IA. The vascular endothelium in normal pregnancy and pre-eclampsia. Reviews of Reproduction. 1996;**1**(2):107-116

[101] Chaiworapongsa T, Romero R, Yoshimatsu J, Espinoza J, Kim YM, Park K, Kalache K, Edwin S, Bujold E, Gomez R. Soluble adhesion molecule profile in normal pregnancy and pre-eclampsia. The Journal of Maternal-Fetal & Neonatal Medicine. 2002;**12**(1):19-27. DOI: 10.1080/jmf.12.1.19.27

[102] Golias C, Tsoutsi E, Matziridis A, Makridis P, Batistatou A, Charalabopoulos K. Leukocyte and endothelial cell adhesion molecules in inflammation focusing on inflammatory heart disease. In Vivo. 2007;**21**(5):757-769

[103] Hidalgo A, Peired AJ, Wild M, Vestweber D, Frenette PS. Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. Immunity. 2007;**26**(4):477-489. DOI: 10.1016/j.immuni.2007.03.011

[104] Schnaar RL, Suzuki A, Stanley P. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. Chapter 31 G-type lectins

[105] Sriramarao P, Berger E, Chambers JD, Arfors KE, Gehlsen KR. High mannose type N-linked oligosaccharides on endothelial cells may influence beta 2 integrin mediated neutrophil adherence in vitro. Journal of Cellular Biochemistry. 1993;**51**(3):360-368. DOI: 10.1002/jcb.240510316

[106] Diamond MS, Staunton DE, Marlin SD, Springer TA. Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. Cell. 1991;**65**(6):961-971

[107] Feduska JM, Garcia PL, Brennan SB, Bu S, Council LN, Yoon KJ. N-glycosylation of ICAM-2 is required for ICAM-2-mediated complete suppression of metastatic potential of SK-N-AS neuroblastoma cells. BMC Cancer. 2013;**13**:261. DOI: 10.1186/1471-2407-13-261

[108] Willhauck-Fleckenstein M, Moehler TM, Merling A, Pusunc S, Goldschmidt H, Schwartz-Albiez R. Transcriptional regulation of the vascular endothelial glycome by angiogenic and inflammatory signalling. Angiogenesis. 2010;**13**(1):25-42. DOI: 10.1007/s10456-010-9162-4

[109] van Kooyk Y, Rabinovich GA. Protein-glycan interactions in the control of innate and adaptive immune responses. Nature Immunology 2008;**9**(6):593-601. DOI: 10.1038/ni.f.203

[110] van Kooyk Y. C-type lectins on dendritic cells: Key modulators for the induction of immune responses. Biochemical Society Transactions 2008;**36**(Pt 6):1478-1481. DOI: 10.1042/BST0361478

[111] Varki A. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. Glycobiology. 2011;**21**(9):1121-1124

[112] Erlebacher A. Immunology of the maternal-fetal interface. Annual Review of Immunology. 2013;**31**:387-411. DOI: 10.1146/annurev-immunol-032712-100003

[113] Laderach DJ, Compagno D, Toscano MA, Croci DO, Dergan-Dylon S, Salatino M, Rabinovich GA. Dissecting the signal transduction pathways triggered by galectin-glycan interactions in physiological and pathological settings. IUBMB Life. 2010;**62**(1):1-13. DOI: 10.1002/iub.281

[114] Nobumoto A, Nagahara K, Oomizu S, Katoh S, Nishi N, Takeshita K, Niki T, Tominaga A, Yamauchi A, Hirashima M. Galectin-9 suppresses tumor metastasis by blocking adhesion to endothelium and extracellular matrices. Glycobiology. 2008;**18**(9):735-744. DOI: 10.1093/glycob/cwn062

[115] Norling LV, Perretti M, Cooper D. Endogenous galectins and the control of the host inflammatory response. The Journal of Endocrinology. 2009;**201**(2):169-184. DOI: 10.1677/JOE-08-0512

[116] Cedeno-Laurent F, Dimitroff CJ. Galectins and their ligands: Negative regulators of anti-tumor immunity. Glycoconjugate Journal. 2012;**29**(8-9):619-625. DOI: 10.1007/s10719-012-9379-0

[117] Macauley MS, Crocker PR, Paulson JC. Siglec-mediated regulation of immune cell function in disease. Nature Reviews. Immunology. 2014;**14**(10):653-666. DOI: 10.1038/nri3737

[118] O'Sullivan JA, Carroll DJ, Bochner BS. Glycobiology of eosinophilic inflammation: Contributions of siglecs, glycans, and other glycan-binding proteins. Frontiers in Medicine. 2017;**4**:116. DOI: 10.3389/fmed.2017.00116

[119] Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. Annual Review of Immunology. 2012;**30**:357-392. DOI: 10.1146/annurev-immunol-020711-075018

[120] Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. Annals of the New York Academy of Sciences. 2012;**1253**:16-36. DOI: 10.1111/j.1749-6632.2012.06517.x

[121] Crocker PR, Varki A. Siglecs in the immune system. Immunology. 2001;**103**(2):137-145

[122] Dam TK, Brewer CF. Maintenance of cell surface glycan density by lectin-glycan interactions: A homeostatic and innate immune regulatory mechanism. Glycobiology. 2010; **20**(9):1061-1064. DOI: 10.1093/glycob/cwq084

[123] Görög P, Pearson JD. Sialic acid moieties on surface glycoproteins protect endothelial cells from proteolytic damage. The Journal of Pathology. 1985;**146**(3):205-212. DOI: 10.1002/path.1711460307

[124] Schauer R. Achievements and challenges of sialic acid research. Glycoconjugate Journal. 2000;**17**(7-9):485-499

[125] Kreisman LS, Cobb BA. Infection, inflammation and host carbohydrates: A glyco-evasion hypothesis. Glycobiology. 2012;**22**(8):1019-1030. DOI: 10.1093/glycob/cws070

[126] Dam TK, Brewer CF. Lectins as pattern recognition molecules: The effects of epitope density in innate immunity. Glycobiology. 2010;**20**(3):270-279. DOI: 10.1093/glycob/cwp186

[127] Rabinovich GA, van Kooyk Y, Cobb BA. Glycobiology of immune responses. Annals of the New York Academy of Sciences. 2012;**1253**:1-15. DOI: 10.1111/j.1749-6632.2012.06492.x

[128] Myatt L, Webster RP. Vascular biology of preeclampsia. Journal of Thrombosis and Haemostasis. 2009;**7**(3):375-384. DOI: 10.1111/j.1538-7836.2008.03259.x

[129] Aird WC. Spatial and temporal dynamics of the endothelium. Journal of Thrombosis and Haemostasis. 2005;**3**(7):1392-1406. DOI: 10.1111/j.1538-7836.2005.01328.x

[130] Bach FH, Robson SC, Ferran C, Winkler H, Millan MT, Stuhlmeier KM, Vanhove B, Blakely ML, van der Werf WJ, Hofer E. Endothelial cell activation and thromboregulation during xenograft rejection. Immunological Reviews. 1994;**141**:5-30

[131] Zhang J, Defelice AF, Hanig JP, Colatsky T. Biomarkers of endothelial cell activation serve as potential surrogate markers for drug-induced vascular injury. Toxicologic Pathology. 2010;**38**(6):856-871. DOI: 10.1177/0192623310378866

[132] Hunt BJ, Jurd KM. Endothelial cell activation. A central pathophysiological process. BMJ. 1998;**316**(7141):1328-1329

[133] Magee LA, Pels A, Helewa M, Rey E, von Dadelszen P. Canadian Hypertensive Disorders of Pregnancy (HDP) Working Group; The hypertensive disorders of pregnancy (29.3). Best Practice & Research. Clinical Obstetrics & Gynaecology. 2015;**29**(5):643-657. DOI: 10.1016/j.bpobgyn.2015.04.001

[134] Laresgoiti-Servitje E. A leading role for the immune system in the pathophysiology of preeclampsia. Journal of Leukocyte Biology. 2013;**94**(2):247-257. DOI: 10.1189/jlb.1112603

[135] Naljayan MV, Karumanchi SA. New developments in the pathogenesis of preeclampsia. Advances in Chronic Kidney Disease. 2013;**20**(3):265-270. DOI: 10.1053/j.ackd.2013.02.003

[136] Ahmed A. New insights into the etiology of preeclampsia: Identification of key elusive factors for the vascular complications. Thrombosis Research. 2011;**127**(Suppl 3):S72-S75. DOI: 10.1016/S0049-3848(11)70020-2

[137] Karumanchi SA, Lindheimer MD. Preeclampsia pathogenesis: "triple a rating"-autoantibodies and antiangiogenic factors. Hypertension. 2008;**51**(4):991-992. DOI: 10.1161/HYPERTENSIONAHA.107.100735

[138] Verlohren S, Muller DN, Luft FC, Dechend R. Immunology in hypertension, preeclampsia, and target-organ damage. Hypertension. 2009;**54**(3):439-443. DOI: 10.1161/HYPERTENSIONAHA.108.120253

[139] Tranquilli AL, Brown MA, Zeeman GG, Dekker G, Sibai BM. The definition of severe and early-onset preeclampsia. Statements from the International Society for the Study of Hypertension in Pregnancy (ISSHP). Pregnancy Hypertens. 2013;**3**(1):44-47. DOI: 10.1016/j.preghy.2012.11.001

[140] Ball E, Bulmer JN, Ayis S, Lyall F, Robson SC. Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. The Journal of Pathology. 2006;**208**(4):535-542. DOI: 10.1002/path.1927

[141] Gun BD, Numanoglu G, Ozdamar SO. The comparison of vessels in elective and spontaneous abortion decidua in first trimester pregnancies: Importance of vascular changes in early pregnancy losses. Acta Obstetricia et Gynecologica Scandinavica. 2006;**85**(4):402-406. DOI: 10.1080/00016340500501731

[142] Lyall F. Priming and remodeling of human placental bed spiral arteries during pregnancy—A review. Placenta. 2005;**26**(Suppl A):S31-S36. DOI: 10.1016/j.placenta.2005.02.010

[143] Espinoza J, Romero R, Mee Kim Y, Kusanovic JP, Hassan S, Erez O, Gotsch F, Than NG, Papp Z, Jai KC. Normal and abnormal transformation of the spiral arteries during pregnancy. Journal of Perinatal Medicine. 2006;**34**(6):447-458. DOI: 10.1515/JPM.2006.089

[144] Burton GJ, Woods AW, Jauniaux E, Kingdom JC. Rheological and physiological consequences of conversion of the maternal spiral arteries for uteroplacental blood flow during human pregnancy. Placenta. 2009;**30**(6):473-482. DOI: 10.1016/j.placenta.2009.02.009

[145] Phipps E, Prasanna D, Brima W, Jim B. Preeclampsia: Updates in pathogenesis, definitions, and guidelines. Clinical Journal of the American Society of Nephrology. 2016;**11**(6):1102-1113. DOI: 10.2215/CJN.12081115

[146] Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. Obstetrical & Gynecological Survey. 2011;**66**(8):497-506. DOI: 10.1097/OGX.0b013e3182331028

[147] Raghupathy R. Cytokines as key players in the pathophysiology of preeclampsia. Medical Principles and Practice. 2013;**22**(Suppl 1):8-19. DOI: 10.1159/000354200

[148] Warrington JP, George EM, Palei AC, Spradley FT, Granger JP. Recent advances in the understanding of the pathophysiology of preeclampsia. Hypertension. 2013;**62**(4):666-673. DOI: 10.1161/HYPERTENSIONAHA.113.00588

[149] Abumaree MH, Chamley LW, Badri M, El-Muzaini MF. Trophoblast debris modulates the expression of immune proteins in macrophages: A key to maternal tolerance of the fetal allograft? Journal of Reproductive Immunology. 2012;**94**(2):131e41. DOI: 10.1016/j.jri.2012.03.488

[150] Abumaree MH, Stone PR, Chamley LW. The effects of apoptotic, deported human placental trophoblast on macrophages: Possible consequences for pregnancy. Journal of Reproductive Immunology. 2006;**72**(1e2):33e45. DOI: 10.1016/j.jri.2006.03.001

[151] Chamley LW, Holland OJ, Chen Q, Viall CA, Stone PR, Abumaree M. Review: Where is the maternofetal interface? Placenta. 2014;**35**(Suppl):S74-S80. DOI: 10.1016/j.placenta.2013.10.014

[152] Shen F, Wei J, Snowise S, DeSousa J, Stone P, Viall C, Chen Q, Chamley L. Trophoblast debris extruded from preeclamptic placentae activates endothelial cells: A mechanism by which the placenta communicates with the maternal endothelium. Placenta. 2014;**35**(10):839-847. DOI: 10.1016/j.placenta.2014.07.009

[153] LaMarca BD, Ryan MJ, Gilbert JS, Murphy SR, Granger JP. Inflammatory cytokines in the pathophysiology of hypertension during preeclampsia. Current Hypertension Reports. 2007;**9**(6):480-485

[154] Saito S, Sakai M. Th1/Th2 balance in preeclampsia. Journal of Reproductive Immunology. 2003;**59**(2):161-173

[155] Daniel Y, Kupferminc MJ, Baram A, Jaffa AJ, Wolman I, Shenhav M, Lessing JB. Plasma soluble endothelial selectin is elevated in women with pre-eclampsia. Human Reproduction. 1998;**13**(12):3537-3541

[156] Fei X, Hongxiang Z, Qi C, Daozhen C. Maternal plasma levels of endothelial dysfunction mediators including AM, CGRP, sICAM-1 and tHcy in pre-eclampsia. Advances in Clinical and Experimental Medicine. 2012;**21**(5):573-579

[157] Farzadnia M, Ayatollahi H, Hasan-Zade M, Rahimi HR. A comparative study of serum level of Vascular Cell Adhesion Molecule-1 (sVCAM-1), Intercellular Adhesion Molecule-1 (ICAM-1) and High Sensitive C-REACTIVE protein (hs-CRP) in normal and pre-eclamptic pregnancies. Iranian Journal of Basic Medical Sciences. 2013;**16**(5):689-693

[158] Haller H, Ziegler EM, Homuth V, Drab M, Eichhorn J, Nagy Z, Busjahn A, Vetter K, Luft FC. Endothelial adhesion molecules and leukocyte integrins in preeclamptic patients. Hypertension. 1997;**29**(1 Pt 2):291-296

[159] Wang Y, Zhang Y, Lewis DF, Gu Y, Li H, Granger DN, Alexander JS. Protease chymotrypsin mediates the endothelial expression of P- and E-selectin, but not ICAM and VCAM, induced by placental trophoblasts from pre-eclamptic pregnancies. Placenta. 2003;**24**(8-9):851-861

[160] Ziganshina MM, Krechetova LV, Vanko LV, Kulterbayeva MA, Sokolyan AV, Sukhikh GT. Time course of changes in the soluble forms of cell adhesion molecules in preeclampsia. Akusherstvo i ginekologiya/Obstetrics and Gynecology (Rus). 2011;(2):42-48

[161] Krauss T, Emons G, Kuhn W, Augustin HG. Predictive value of routine circulating soluble endothelial cell adhesion molecule measurements during pregnancy. Clinical Chemistry. 2002;**48**(9):1418-1425

[162] Szarka A, Rigó J Jr, Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunology. 2010;**11**:59. DOI: 10.1186/1471-2172-11-59

[163] Veas CJ, Aguilera VC, Muñoz IJ, Gallardo VI, Miguel PL, González MA, Lamperti LI, Escudero CA, Aguayo CR. Fetal endothelium dysfunction is associated with circulating maternal levels of sE-selectin, sVCAM1, and sFlt-1 during pre-eclampsia. The Journal of Maternal-Fetal & Neonatal Medicine. 2011;**24**(11):1371-1377. DOI: 10.3109/14767058.2011.556204

[164] Robajac D, Vanhooren V, Masnikosa R, Miković Ž, Mandić V, Libert C, Nedić O. Preeclampsia transforms membrane N-glycome in human placenta. Experimental and Molecular Pathology. 2016;**100**(1):26-30. DOI: 10.1016/j.yexmp.2015.11.029

[165] Sukhikh GT, Ziganshina MM, Nizyaeva NV, Kulikova GV, Volkova JS, Yarotskaya EL, Kan NE, Shchyogolev AI, Tyutyunnik VL. Differences of glycocalyx composition in the structural elements of placenta in preeclampsia. Placenta. 2016;**43**:69-76. DOI: 10.1016/j.placenta.2016.05.002

[166] Stern R, Asari AA, Sugahara KN. Hyaluronan fragments: An information-rich system. European Journal of Cell Biology. 2006;**85**(8):699-715. DOI: 10.1016/j.ejcb.2006.05.009

[167] Frey H, Schroeder N, Manon-Jensen T, Iozzo RV, Schaefer L. Biological interplay between proteoglycans and their innate immune receptors in inflammation. The FEBS Journal. 2013;**280**(10):2165-2179. DOI: 10.1111/febs.12145

[168] Marini M, Bonaccini L, Thyrion GD, Vichi D, Parretti E, Sgambati E. Distribution of sugar remains in human placentas from pregnancies complicated by hypertensive disorders. Acta histochemica. 2011;**113**:815-825. DOI: 10.1016/j.acthis.2010.12.001

[169] Berg S, Engman A, Holmgren S, Lundahl T, Laurent TC. Increased plasma hyaluronan in severe pre-eclampsia and eclampsia. Scandinavian Journal of Clinical and Laboratory Investigation. 2001;**61**(2):131-137

[170] Romão M, Weel IC, Lifshitz SJ, Peraçoli MT. Elevated hyaluronan and extracellular matrix metalloproteinase inducer levels in women with preeclampsia. Archives of Gynecology and Obstetrics. 2014;**289**(3):575-579. DOI: 10.1007/s00404-013-3021-7

[171] Hentschke MR, Lucas LS, Mistry HD, Pinheiro da Costa BE, Poli-de-Figueiredo CE. Endocan-1 concentrations in maternal and fetal plasma and placentae in pre-eclampsia in the third trimester of pregnancy. Cytokine. 2015;**74**(1):152-156. DOI: 10.1016/j.cyto.2015.04.013

[172] Cakmak M, Yilmaz H, Bağlar E, Darcin T, Inan O, Aktas A, Celik HT, Ozdemir O, Atalay CR, Akcay A. Serum levels of endocan correlate with the presence and severity

of pre-eclampsia. Clinical and Experimental Hypertension. 2016;**38**(2):137-142. DOI: 10.3109/10641963.2015.1060993

[173] Adekola H, Romero R, Chaemsaithong P, Korzeniewski SJ, Dong Z, Yeo L, Hassan SS, Chaiworapongsa T. Endocan, a putative endothelial cell marker, is elevated in pre-eclampsia, decreased in acute pyelonephritis, and unchanged in other obstetrical syndromes. The Journal of Maternal-Fetal & Neonatal Medicine. 2015;**28**(14):1621-1632. DOI: 10.3109/14767058.2014.964676

[174] Siddiqui MF, Nandi P, Girish GV, Nygard K, Eastabrook G, de Vrijer B, Han VK, Lala PK. Decorin over-expression by decidual cells in preeclampsia: A potential blood biomarker. American Journal of Obstetrics and Gynecology. 2016;**215**(3):361.e1-361.e15. DOI: 10.1016/j.ajog.2016.03.020

[175] Khedun SM, Naicker T, Moodley J, Gathiram P. Urinary heparin sulfate proteoglycan excretion in black African women with pre-eclampsia. Acta Obstetricia et Gynecologica Scandinavica. 2002;**81**(4):308-312

[176] Rabelink TJ, de Zeeuw D. The glycocalyx-linking albuminuria with renal and cardiovascular disease. Nature Reviews. Nephrology. 2015;**11**(11):667-676. DOI: 10.1038/nrneph.2015.162

[177] Kim YH, Nijst P, Kiefer K, Tang WH. Endothelial glycocalyx as biomarker for cardiovascular diseases: Mechanistic and clinical implications. Current Heart Failure Reports. 2017;**14**(2):117-126. DOI: 10.1007/s11897-017-0320-5