Diploma Thesis

Preeclampsia and smoking, and their effects on the retinal microvasculature – a literature review

submitted by

Moritz Dohrmann

to attain the academic degree

Doctor medicinae universae

(Dr. med. univ.)

at the

Medical University of Graz

conducted at

Department of Physiology

under supervision of

Assoz. Prof. Priv.-Doz. Dr.med.
Dr.med.univ. MMedSci PhD Nandu Goswami
Ao.Univ.-Prof. Dr.med.univ. Mila Cervar-Zivkovic

Graz, 22.5.2022

Statutory declaration

I hereby declare that I have authored this thesis independently and without any support from third parties, that I have not used other than the explicitly marked sources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, 22.5.2022 Moritz Dohrmann eh

Acknowledgements

Foremost, I would like to thank my supervisor Nandu Goswami for giving me this opportunity and continuously supporting me throughout the project. Moreover, I am grateful for the insightful comments during our time in the laboratory.

In addition, I would like to thank the Medical University of Graz and its staff for providing me with an access to research facilities and technical assistance.
Last but not least, I would like to thank my sister for proofreading of my thesis and my parents and my girlfriend for their love and encouragement throughout my studies.

Table of contents

Abbreviations

ACE	angiotensin converting enzyme
ADH	anti-diuretic hormone
ADM	adrenomedullin
AVR	arteriovenous ratio
BMI	body mass index
CI	confidence interval
CRAE	central retinal arteriolar equivalent
cCRAE	corrected central retinal arteriolar equivalent
CRVE	central venous arteriolar equivalent
cCRVE	corrected central venous arteriolar equivalent
CO	carbon monoxide
CSE	cigarette smoke extract
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
HELLP-syndrome	hemolysis, elevated liver, low platelet syndrome
IUGR	intrauterine growth restriction
LSCI	laser speckle contrast imaging
NKs	natural killer cells
OR	odd ratio
PE	preeclampsia
PlGF	placental growth factor
RAAS	renin-angiotensin-aldosterone-system
RR	relative risk
sEng	soluble endoglin
sFLT1	soluble FMS-like tyrosinkinase 1 receptor
SVR	systemic vascular resistance
TGF-β1	transforming growth factor β1
VEGF	vascular endothelial growth factor

Index of figures

https://www.researchgate.net/figure/Retinal-vascular-calibre-measurement-and-Retinopathy-Retinal-arteriolar-and-venular_fig3_273181510

Figure 7

Selection process of studies included in the review

Figure 8

Relationship between the retinal microvasculature, smoking and PE

Figure 9

Comparing changes in angiogenic and antiangiogenic factors between smoking and PE

Index of tables

Zusammenfassung in Deutsch

Hintergrund:
Präeklampsie, eine weiterhin schwerwiegende Komplikation in der Schwangerschaft, verändert die vaskoendotheliale Gesundheit bei schwangeren Frauen, was zu Bluthochdruck und Organfunktionsstörungen führt. Es hat sich gezeigt, dass das Rauchen von Zigaretten ebenfalls negative Auswirkungen auf die Gefäßfunktionen hat. Wie sich das Zigarettenrauchen auf die Mikrogefäße bei Frauen mit hohem Risiko für Präeklampsie auswirkt, ist jedoch nicht bekannt. Die Fotografie der retinalen Mikrovaskulatur kann verwendet werden, um Veränderungen der Mikrovaskulatur zu erkennen, und kann ein nützliches Werkzeug zu sein, um die Veränderungen der Mikrovaskulatur zu vergleichen, die durch Präeklampsie und Rauchen verursacht werden.

Zielsetzung:
Einen Überblick über ausgewählte Studien zu geben, die den Zusammenhang zwischen Rauchen oder Präeklampsie und der retinalen Mikrovaskulatur untersuchen, um die möglichen Zusammenhänge zwischen beiden zu verstehen und die Mechanismen hervorzuheben, durch die sie zusammenwirken.

Methodik:
Wir haben den Ansatz einer narrativen Literaturrecherche verwendet. Mit den ermittelten Schlüsselwörtern wurde auf PubMed nach geeigneten Artikeln gesucht.

Ergebnisse:
Insgesamt wurden 13 Studien nach Screening und Auswertung in die Übersicht eingeschlossen. Hinweise deuten stark auf eine paradoxerweise positive Beziehung zwischen Rauchen und dem Risiko für Präeklampsie hin, die es signifikant reduziert. Die Studien zeigen eine Verengung des Kalibers der Netzhautgefäße bei Frauen mit PE und dagegen eine Erweiterung des Kalibers der Netzhautgefäße bei Raucherinnen. Angiogene Faktoren und Kohlenmonoxid können einige der Mechanismen sein, die an der paradoxen Beziehung beteiligt sind.

Schlussfolgerungen:
In diesem Review soll das Rauchen während der Schwangerschaft nicht befürwortet werden, da die Nebenwirkungen den Schutz vor Präeklampsie immer noch überwiegen. Die gegenläufigen Wirkungen auf das Mikrogefäßsystem

könnten für die Schutzwirkung des Rauchens verantwortlich sein, bedürfen jedoch weiterer Untersuchungen. Die Therapie der Präeklampsie könnte sich mit dem Wissen um die Mechanismen ändern und eine frühere Diagnose mit Hilfe der Netzhautfotografie erfolgen.

Abstract

Background:

Preeclampsia, remaining a severe complication in pregnancies, alters the vasco-endothelial health in pregnant women causing hypertension and organ dysfunction. Cigarette smoking has been shown to have adverse effects on vascular functions as well. However, how cigarette smoking affects the microvasculature in women with high risk of pre-eclampsia is not well known. Retinal microvasculature photography can be used to detect changes to the microvasculature and seems like a useful tool to compare the changes in the microvasculature caused by preeclampsia and smoking.

Aims:

To provide an overview of selected studies investigating the relation of smoking or preeclampsia and the retinal microvasculature to understand the possible associations between both and to highlight the mechanisms by which they correlate.

Methodology:

We used the approach of a narrative literature review. Search for suitable articles was committed on PubMed with the determined key words.

Results:

In total 13 studies were included in the review after screening and evaluating. Evidence strongly hints at a paradoxically positive relation between smoking and the risk for preeclampsia reducing it significantly. The studies show a narrowing of the retinal vessel calibre in women with PE and oppositional a widening in the calibre of the retinal vessels in people who smoke. Angiogenic factors and carbon monoxide may be some of the mechanisms involved in the paradox relation.

Conclusions:

This review is not supposed to advocate smoking during pregnancy as the adverse effects still outweigh the protection from preeclampsia. The counteracting effects on the microvasculature might be responsible for the protective effect of smoking but needs more investigation. Therapy of preeclampsia might change with the knowledge of the mechanisms and earlier diagnosis might be performed with the help of retinal photography.

1 Introduction

Preeclampsia (PE) has been the subject to a lot of research. In past studies, mostly epidemiological studies, animal models and in-vitro experiments have been used to get a better understanding of the development of preeclampsia, its genesis, and its progress. The pathomechanisms as well as all the possible causes of PE are yet to be fully discovered. Understanding the underlying causes for PE and evaluating their risks to develop into the disease crucially contribute to the prevention and therapy of PE.

Smoking, on the other hand, is well known for its negative long-term effects on the human body with cigarette smoke containing an abundance of toxic ingredients. Smoking during pregnancy is generally established as highly toxic to the child regarding its development and its delivery, through intrauterine dystrophy, spontaneous abortion, or placental abruption.

Strong evidence suggests an increase in adverse pregnancy outcomes linked to smoking. In Europe around 8% of women smoke during their pregnancy (Lange *et al.*, 2018), making it continuously relevant to increase knowledge on the effect it can have on mother and child.

The effect of smoking on preeclampsia is still not fully known. This literature review will go deeper on the impact of smoking, by reviewing the latest studies on this topic.

1.1 Regulation of blood pressure

High blood pressure and its correlated diseases remain one of the highest mortality rates in the industrial world, therefore a lot of research has been conducted to understand the pathways causing it and to create therapies to inhibit its progression.

The systemic blood pressure is determined by the cardiac output and the systemic vascular resistance (SVR). Changes in one or both factors result in changes of the systemic blood pressure. The two factors are regulated and adapt all the time physiologically depending on the need or can be changed pathologically resulting

in hyper- or hypotension. Physiological blood pressure regulation is a complex chemical and neuronal machinery and can be divided into short- and long-term mechanisms.

The short-term blood pressure regulation, which plays an important role in balancing fluctuations of blood pressure through orthostatic changes or changes in volume, is controlled mainly by the autonomic nervous system. The fluctuations are detected by baroreceptors in the carotid sinus and the arch of the aorta. A rise in blood pressure causes the walls of these vessels to be stretched, and therefore the baroreceptors to send signals to the solitary nucleus in the brainstem (Shahoud, Sanvictores and Aeddula, 2022), which in response inhibits the sympathetic response of the autonomic nervous system (Sanders, Mark and Ferguson, 1989). There are also low pressure baroreceptors mainly in the venous system that can detect changes in volume and in case of hypovolaemia lead to an increase in anti-diuretic hormone, renin and aldosterone (Shahoud, Sanvictores and Aeddula, 2022).

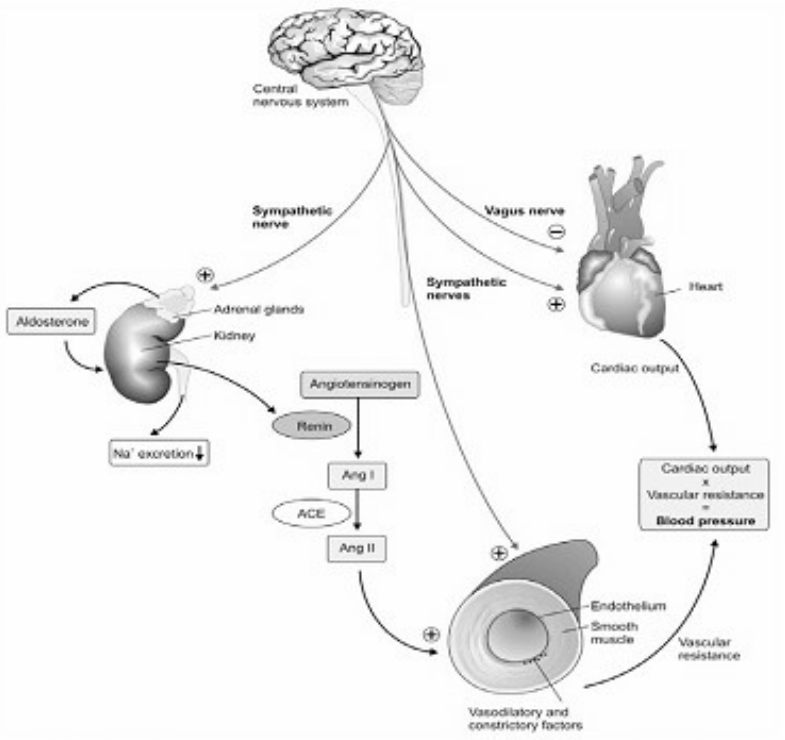

Figure 1: Overview of systems involved in blood pressure regulation. Reproduced from: https://www.researchgate.net/profile/Mithun-Bhowmick-2/publication/317258567/figure/fig2/AS:500007146975233@1496222401632/A-schematic-diagram-of-the-blood-pressure-regulation-system-Management-of-Hypertension.png

Long-term blood pressure regulation relies stronger on endocrine mechanisms. The main intermediators are the Renin-Angiotensin-Aldosterone-System (RAAS) and the anti-diuretic hormone (ADH).
The RAAS starts with the release of Renin from the juxtaglomerular cells in the afferent arterioles of the kidney, which are stimulated by hypoperfusion and sympathetic input. This sets of a signalling cascade where Renin converts Angiotensinogen into Angiotensin I, which further converts with the help of the Angiotensin-converting-Enzyme (ACE) into Angiotensin II. Angiotensin II induces vasoconstriction, increasing the systemic resistance and therewith the blood pressure. It also increases blood pressure by retention of water and sodium both directly in the kidney (Ichikawi and Harris, 1991) and indirectly through stimulating the adrenal cortex to release Aldosterone.
The other pathway is the anti-diuretic hormone or vasopressin. It is produced in the hypothalamus and released from the pituitary gland, when triggered by low blood pressure, low volume and by Angiotensin II also and it increases the reabsorption of water in the kidney. Oppositional the atrial natriuretic peptide, released from the atria through high blood pressure, causes an increase in the glomerular filtration rate (GFR) and excretion of sodium resulting in lower blood pressure.

During the pregnancy the cardiovascular system of the mother changes and adapts to the new circumstances, therefore in a healthy pregnancy the blood pressure remains quite stable, slightly decreasing in the beginning of the pregnancy and then increasing until the delivery before returning to a normal level. This is due to the systemic vascular resistance decreasing while mutually the cardiac output and the blood volume increase to ensure the increased demands of blood flow to the uterine artery (Chapman *et al.*, 1998).

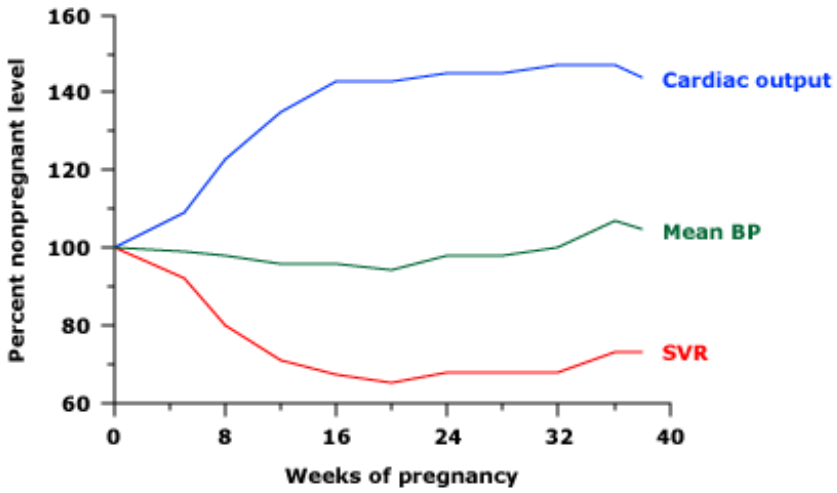

Figure 2: Changes in blood pressure, SVR and cardiac output during pregnancy. Reproduced from: UpToDate Graphic 54685 Version 8.0 https://www-1uptodate-1com-1wwbz631a0030.han.medunigraz.at/contents/image?imageKey=NEPH%2F54685&topicKey=OBGYN%2F443&search=blood%20pressure%20pregnancy&source=outline_link&selectedTitle=3~150

1.2 Preeclampsia

Preeclampsia is a pregnancy related hypertensive disorder that is defined by de novo appearing of hypertension ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic (after the 20th week gestational age) and proteinuria (above 0.3 g/24h or Protein/Creatinine ratio > 30 mg/mmol) or de novo hypertension and severe organ dysfunction ('Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222', 2020). It can also occur in women with chronic hypertension as a superimposed form of PE with an increase in blood pressure or the development of proteinuria or severe organ dysfunction.

Undetected and untreated the disease can further complicate into eclampsia, resulting in grand-mal seizures, or the HELLP-syndrome, both with severe and possibly even lethal consequences for the mother. On the fetal side complications like intrauterine growth restriction (IUGR), oligohydramnios or abruptio placentae may occur, putting the unborn child's life at risk by possibly leading to an indication for preterm delivery or abortion.

In the diagnosis of PE one can differentiate a severe form of PE, which is characterized by PE in addition with one or more of the following features:

- Systolic blood pressure elevation ≥ 160mmHg or diastolic blood pressure elevation ≤ 110mmHg
- Thrombocytopenia (platelet count <100,000/μL)
- Pulmonary edema
- Impaired liver function and/or persistent epigastric pain
- Renal abnormality, increasing renal insufficiency
- Neurological symptoms like strong headaches or visual impairment

PE can also be divided by time of appearance into early-onset (before 34 weeks gestational age) and late-onset (after 34 weeks gestational age). Newer studies show that early-onset preeclampsia is rather associated with immunological aetiology, whereas late-onset preeclampsia is contributed with maternal vascular predispositions like chronic hypertension (Robillard *et al.*, 2019).

1.2.1 Epidemiology

"Preeclampsia remains a leading cause of maternal and perinatal mortality and morbidity" (Steegers *et al.*, 2010). The incidence of preeclampsia worldwide is estimated to be 2% to 8% (Steegers *et al.*, 2010) with a wide variety depending on the geographics. The mortality of PE depends largely on the accessibility of prenatal maternal care. Countries that are with a generally good health care system have a lesser mortality, compared with poorer countries. Overall around 10% to 15% of all maternal deaths are associated with PE or eclampsia (Duley, 2009).

Hypertensive disorders during pregnancies are responsible for the most maternal death in Latin America and the Caribbean with an average covering of 25.7% of all maternal deaths. (Khan *et al.*, 2006) In developed countries PE has a lesser mortality, being responsible for 16% of maternal deaths (Khan *et al.*, 2006) but the morbidity is, as is with all hypertensive disorders, still relatively high, and rising as a study by Wallis et al, from 2008 suggests, showing an increase in the age-adjusted incidence of preeclampsia and gestational hypertension from 1987 – 2004 in the United States (Wallis *et al.*, 2008). Even further the risk for a severe case of PE has increased 6.7-fold from 1980 to 2003. Those findings have been

linked to an increase in the average maternal age and the increase in obesity (Stevens *et al.*, 2017). The estimated numbers for Europe name an incidence of 5.3% but the national data availability is currently scarce (Abalos *et al.*, 2013).
Besides geographical differences, socioeconomic status and race also affect the outcome of the disease, as shows a study by Ross et al., which associated a higher socioeconomic status with a lower risk for preeclampsia and longer gestation in white American women, in adverse to black American women, whose risk was unaffected by their socioeconomic status, but higher in general (Ross *et al.*, 2019).

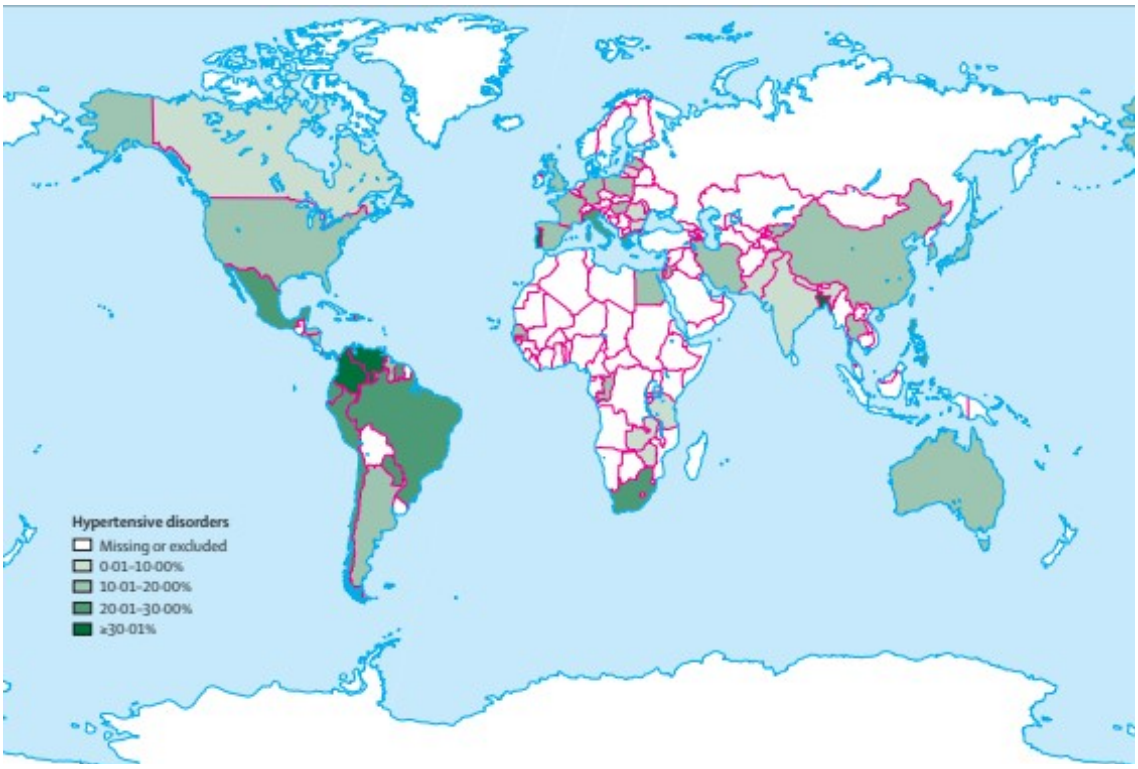

Figure 3: Percentage of maternal mortality of hypertensive pregnancy disorders around the world. Reproduced from Khan et al., 2006

1.2.2 Differential diagnosis and other hypertensive pregnancy disorders

PE is part of a group of pregnancy related hypertensive disorders which also include gestational hypertension, superimposed chronic hypertension, the HELLP-

syndrome, and lastly eclampsia. Their common main symptom is hypertension, but their causes can differ. In practical medicine it is important to distinguish these hypertensive diseases to give the optimal treatment for the becoming mother.

Chronic hypertension exists before the pregnancy, however it is a major risk factor and PE can develop superimposed upon it often leading to a more severe form of the disease.

In contrast gestational hypertension is a de novo appearing hypertension during pregnancy without the other features of PE like proteinuria or severe organ dysfunction, but it can still develop into PE during the course of pregnancy and should be checked on regularly.

The HELLP-Syndrome is suggested to be a special, more severe subtype of preeclampsia, occurring “in 0.5% to 0.9% of all pregnancies and in 10% to 20% of cases with severe preeclampsia” (Haram, Svendsen and Abildgaard, 2009; Geary, 1997). The acronym HELLP describes the symptoms of hemolysis, significant elevated liver enzymes and low platelets (thrombocytopenia; thrombocytes <100,000/µL)

Eclampsia lastly describes the fully developed end stage symptoms of PE with the main symptom being grand mal seizures. It is associated with highly dangerous and possibly lethal maternal, obstetric, and neonatal complications and requires immediate medical intervention.

Chronic Hypertension	Hypertension diagnosed or present before pregnancy or before 20 weeks of gestation. Hypertension that is first diagnosed during pregnancy and persists for at least 12 weeks post-delivery is also considered chronic hypertension
Gestational Hypertension	New onset of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on at least 2 occasions 4 hours apart after 20 weeks of gestation in a previously normotensive individual
Preeclampsia	New onset of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg on at least 2 occasions at least 4 hours apart after 20 weeks of gestation in a previously normotensive individual **or** systolic blood pressure ≥160 mmHg

	or diastolic blood pressure ≥110 mmHg confirmed within a short interval (minutes) to facilitate timely antihypertensive therapy **And:** - Proteinuria (≥300 mg per 24-hour urine collection [or this amount extrapolated from a timed collection], or protein:creatinine ratio ≥0.3, or urine dipstick reading ≥2+ [if other quantitative methods are not available]) **Or, in the absence of proteinuria:** - Thrombocytopenia (platelet count <100,000/μL) - Renal insufficiency (serum creatinine of >1.1 mg/dL [97 μmol/L] or a doubling of the serum creatinine concentration in the absence of other renal disease) - Impaired liver function as indicated by liver transaminase levels at least twice the normal concentration - Pulmonary edema - Persistent cerebral or visual symptoms
Preeclampsia with severe features	**Any of these findings in a patient with preeclampsia:** - Systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg on 2 occasions at least 4 hours apart (unless antihypertensive therapy is initiated before this time) - Thrombocytopenia (platelet count <100,000/μL) - Impaired liver function as indicated by liver transaminase levels at least twice the normal concentration or severe persistent right upper quadrant or epigastric pain unresponsive to medication and not accounted for by alternative diagnoses, or both - Progressive renal insufficiency (serum creatinine concentration >1.1 mg/dL [97 μmol/L] or a doubling of the serum creatinine concentration in the absence of other renal disease) - Pulmonary edema - Persistent cerebral or visual disturbances
HELLP-	Presence of Hemolysis, Elevated Liver enzymes, and Low

Syndrome	Platelet count; hypertension may be present (HELLP in such cases is often considered a variant of preeclampsia)
Eclampsia	In a patient with preeclampsia, generalized seizures that cannot be attributed to other causes

Table 1: Definitions of hypertensive pregnancy disorders. Adapted from UpToDate, Graphic 127246 Version 3.0
https://www-1uptodate-1com-1wwbz631a0030.han.medunigraz.at/contents/image?imageKey=OBGYN%2F127246&topicKey=OBGYN%2F6814&search=preeclampisa&rank=1~150&source=see_link

1.2.3 Risk factors

There are several practical clinical risk factors that, either alone or in combination, might identify women in early pregnancy who are at “high risk” of developing pre-eclampsia. Most noticeably a prior event of preeclampsia (relative risk 8.4%, 95% CI 7.1% to 9.9%), chronic hypertension (relative risk 5.1%, 95% CI 4.0% to 6.5%) and pregestational diabetes (relative risk 3.7%, 95% CI 3.1% to 4.3%) (Bartsch *et al.*, 2016).

There are other known factors increasing the relative risk of preeclampsia, which are a multifetal pregnancy, nulliparity, advanced maternal age, a family history of preeclampsia, kidney disease, increased body mass index (BMI), prior IUGR, prior placental abruption and prior stillbirth, as well as the use of assisted reproductive technology which is also acknowledged to be a risk factor (Bartsch *et al.*, 2016).

Patients with autoimmune disease, like the antiphospholipid syndrome and systemic lupus erythematosus, also have increased morbidity with a relative risk of 2.8% (95% CI 1.8%-4.3%) and 1.8% (95% CI 1.5%-2.1%) respectively (Bartsch *et al.*, 2016), though the pathophysiological reason are yet to be fully explored.

The increased risk with a positive family history also suggests PE to be caused by a genetical disposition which could be derived by both the mother as well as the father (Esplin *et al.*, 2001). Several genetical polymorphisms that might affect the development of PE have been found already but need to be researched more thoroughly (Lachmeijer *et al.*, 2002).

1.2.4 Pathophysiology of Preeclampsia

Pathophysiology of PE is still an object to scientific research as its multifactorial pathways are still in need of being explored thoroughly. The research in animals, though very needed, proves challenging since only humans are observed developing PE unaffiliated, and the animal models are only designed to imitate the disease for instance by artificially reducing the blood flow to the placenta (Rana *et al.*, 2019).

The pathogenesis of preeclampsia progresses in two stages, first is the abnormal placentation during the early first trimester of pregnancy, followed by a maternal syndrome in the later second and third trimester characterized by an excess of antiangiogenic factors, that result in inflammation, endothelial dysfunction and maternal systemic disease. (Roberts *et al.*, 1989; Redman and Sargent, 2005; Romero and Chaiworapongsa, 2013).

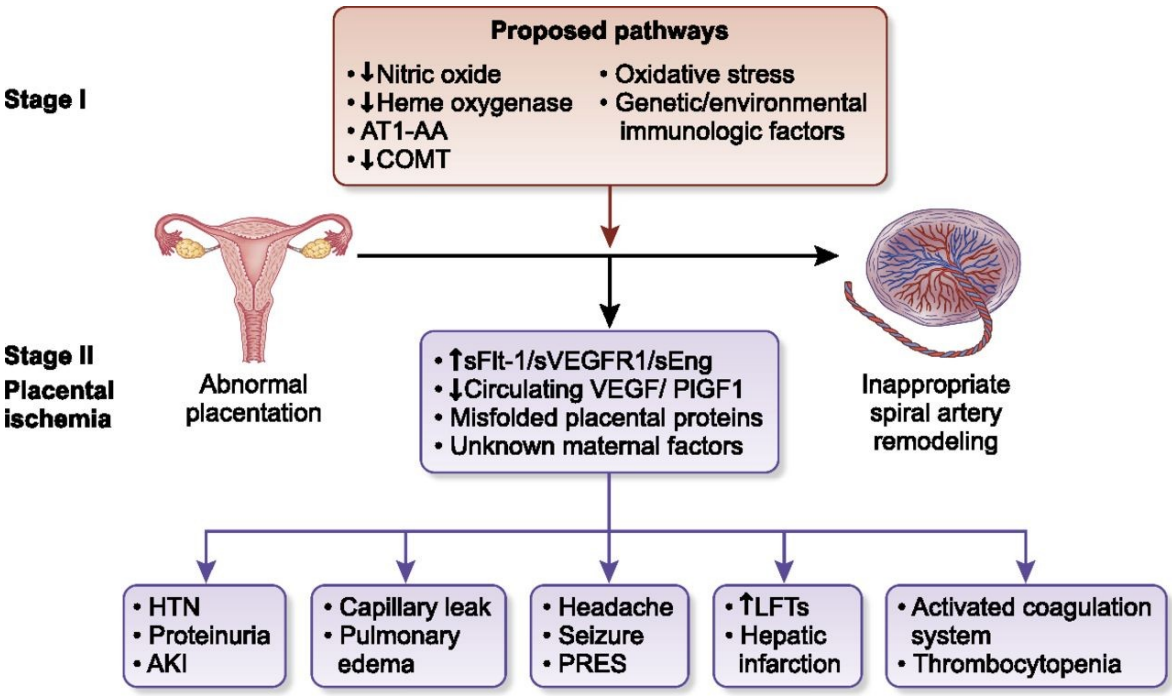

Figure 4: Overview of the pathogenesis of PE. Reproduced from: https://cjasn.asnjournals.org/content/clinjasn/11/6/1102/F1.large.jpg?width=800&height=600&carousel=1

In the first stage, the pathological placentation, the cytotrophoblast fails to change from a epithelial proliferative state into a endothelial invasive state and properly invade into the decidua and transform the spiral arteries of the mother (Zhou,

Damsky and Fisher, 1997) and therefore causing placental hypoperfusion and ischemia (Brosens and Renaer, 1972). The reasons for this abnormal placentation are still being discussed, a number of possible causes have been proposed, including oxidative stress, abnormal natural killer cells (NKs) at the maternal-fetal interface and genetic and environmental factors, though none of the theories have conclusive evidence in humans yet (Rana *et al.*, 2019).

The uteroplacental ischemia in response leads to the systemic maternal reaction with an increase in blood pressure and the symptoms of multi-organ failure assigned with preeclampsia (Palei *et al.*, 2013), by releasing soluble factors, such as soluble FMS-like tyrosinkinase 1 receptor (sFLT1) and soluble endoglin (sEng), in the mothers circulation (Rana *et al.*, 2019). These factors are usually found in the cell walls of endothelial cells, but in patients with PE they are embedded in syncytial debris, circulating in the system of the mother (Germain *et al.*, 2007; Rajakumar *et al.*, 2012). Both sFLT1 and sEng are soluble receptor-proteins which exert their antiangiogenic effects by binding and prohibiting of proangiogenic proteins (Powe, Levine and Karumanchi, 2011) such as the vascular endothelial growth factor (VEGF) and its homolog form the placental growth factor (PlGF) bound by sFLT1 and the transforming growth factor β1 (TGF-β1) which is bound by sEng (Venkatesha *et al.*, 2006).

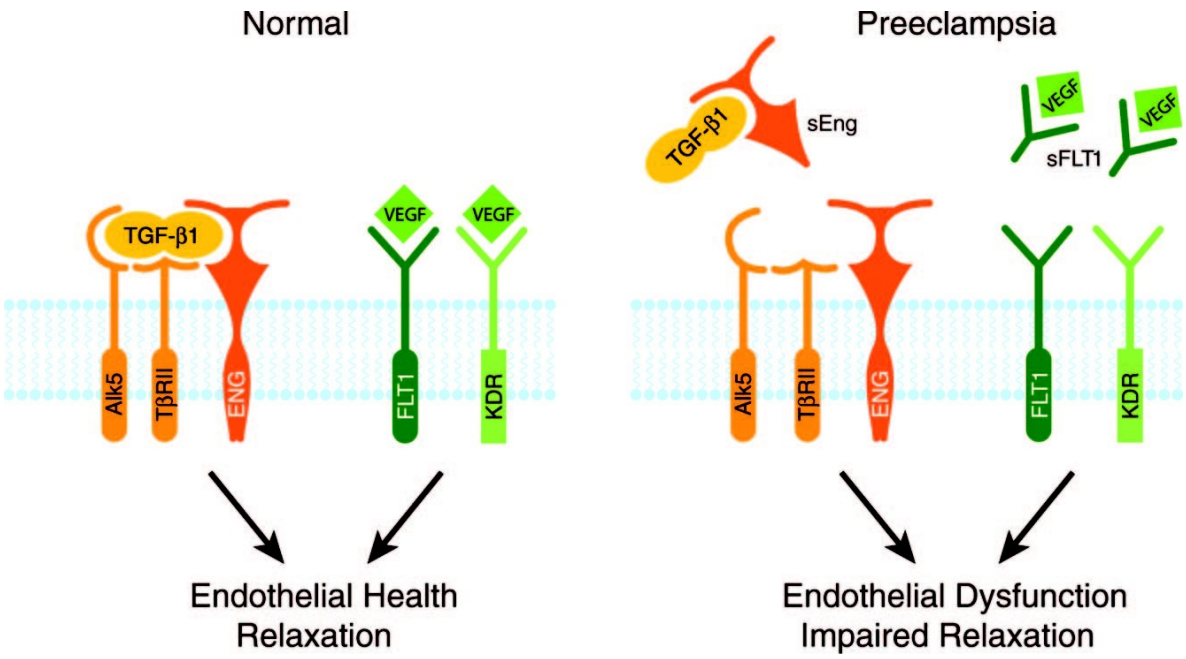

Figure 5: Showing the disturbed pathway of endothelial factors in PE compared to normal. Reproduced from Powe et al., 2011

The rise of sEng and the soluble form of vascular cell adhesion molecule 1 (sVCAM-1), as well as a fall of VEGF has been observed in women with PE with severe features. Adhesion molecules play an important role in the mediation of leucocytes, their soluble form may hint at increased vascular dysfunction (Rios *et al.*, 2016).

Additionally, a rise in cell free placental DNA has been observed in women developing PE which originates from the apoptotic and necrotic placental cells and may contribute to the systemic inflammation (Levine *et al.*, 2004; Hartley, Ferguson and Moffett, 2015).

The manifestation of the clinical symptoms of PE, such as high blood pressure, oedema and proteinuria as well as the organ dysfunction, can be derived as an effect of the endothelial dysfunction and has been linked to uteroplacental ischemia in animal models (Palei *et al.*, 2013).

1.3 The retinal photography as means to measure microvasculature

Fundus photography is a relatively modern approach to measure human microvasculature non-invasive and in-vivo. In recent years, the technology of fundus photography has developed to a point, that it is possible to take detailed pictures of the retinal fundus of the human eye to analyse the morphological changes in the retinal microvasculature. This usage of the technology has a promising range of application. Its original use lays in the diagnosis of ophthalmological diseases, but its non-invasive approach to the retinal vessels has made it an increasingly important tool in the research on microvasculature development. The microvasculature consists of blood vessels with a diameter of less than 150 µm and play an important role in maintaining cardiovascular health (De Boever *et al.*, 2014). When compared, the microvasculature of the retina shows similar traits to the one in other tissues, such as heart and brain (De Silva *et al.*, 2011).

Retinal photography has allowed for new insights in the development of microvascular changes, that are often the predecessors of systemic cardiovascular disease, and are subtle to be affected by lifestyle and

environmental factors including smoking (Sun *et al.*, 2009; Serre and Sasongko, 2012).

The procedure of taking the picture is done in 5 minutes and is suitable for all age groups, making it an efficient tool in epidemiological studies. After this the retinal images are analysed with help of the IVAN software to determine the central retinal arteriolar equivalent (CRAE) and the central retinal venular equivalent (CRVE). These values are calculated by using the revised formulas of Parr and Hubbard, taking the calibre of the six largest vessels for both arterioles and venules into account and summarizing them to get the correspondent central retinal equivalent (Knudtson *et al.*, 2003).

Formula for CRAE arterioles: $\widehat{W} = 0.88 * \left(w_1^2 + w_2^2\right)^{1/2}$

Formula for CRVE venules: $\widehat{W} = 0.95 * \left(w_1^2 + w_2^2\right)^{1/2}$

The central retinal equivalents allow for an objective comparison between the general vessel calibres of retinal microvasculature. DeBoever et al. did several repeated measurements of CRAE and CRVE to assess their objective value and estimated them as highly reliable values (De Boever *et al.*, 2014).

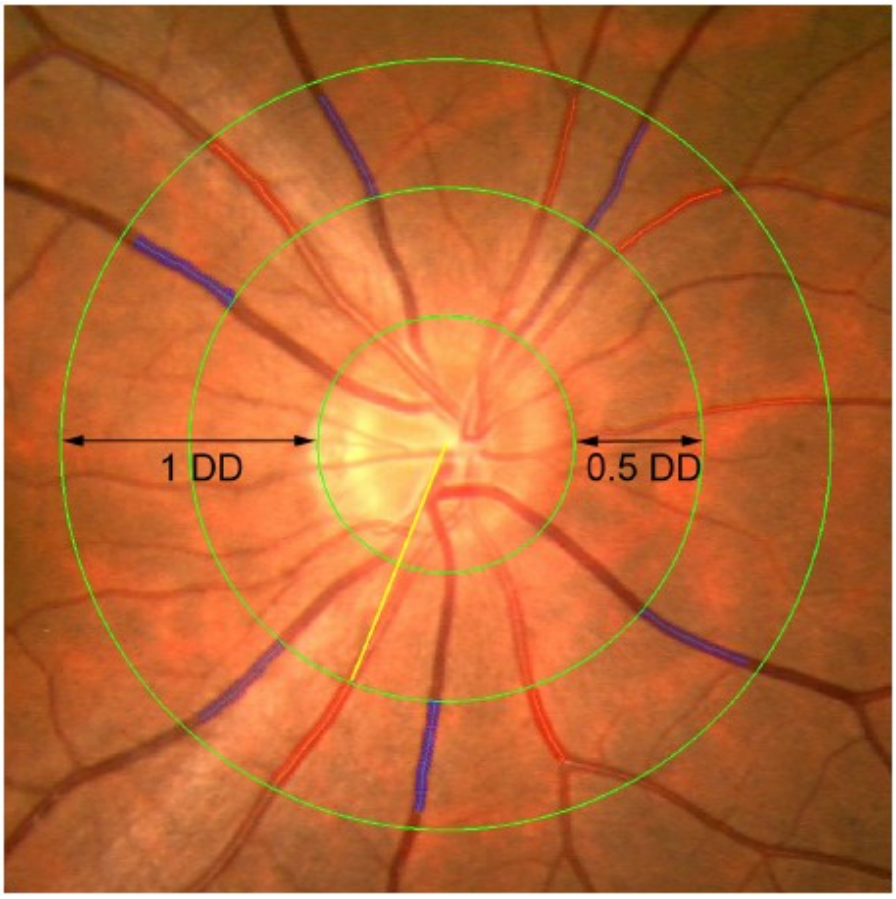

Figure 6: Retinal image of the optical disc and surrounding area used to evaluate retinal microvasculature. Highlighted are the six largest branches of arterioles (red) and venules (blue), measurements are taken within 0.5 and 1 optical disc diameters away from the optical nerve head (green circles). Reproduced from: https://www.researchgate.net/figure/Retinal-vascular-calibre-measurement-and-Retinopathy-Retinal-arteriolar-and-venular_fig3_273181510

Another often used figure in the analysing of retinal microvasculature is the ratio between diameters of retinal arterioles to retinal venules called the arteriovenous ratio (AVR) which indicates arteriolar narrowing when decreased. There are several formulas for determination of the AVR. According to Hemminki et al. the most suitable ones are the value of CRAE divided by the value of CRVE (CRAE/CRVE) or the sum of squares of widths of arterioles divided by the sum of squares of widths of venules (Hemminki *et al.*, 2007).

In the research on PE retinal vessels may be ideally suited as a model to study changes to the microvasculature during pregnancy, as neither the placenta nor the retinal blood vessels are innervated by the autonomic nervous system (Funk, 1997; Marzioni *et al.*, 2004; Lupton, Chiu, Hodgson, Tooher, Lujic, *et al.*, 2013).

Visual symptoms that may occur under PE show that the retinal microvasculature can forfeit to the disease.

2 Aims and objectives

This thesis, reviewing current literature on the topic, is supposed to get an understanding of the microvascular changes occurring under different circumstances and smoking behaviours in pregnant women and perhaps to help in finding a hypothesis for future practical clinical studies in this area. In its introduction it is also supposed to summarize some pathogenetic processes of preeclampsia as well as describe the role of retinal imaging in microvasculature research and early diagnosis of the illness before clinical manifestation.

3 Search methodology

Originally the thesis was meant to be a prospective clinical study measuring the calibres of retinal microvasculature in pregnant women who either smoked, had been smoking until pregnancy and ceased, or never smoked and compare the results. Unfortunately, due to restrictions due to the Covid19 this could not be accomplished.

The thesis was then carried out as a literature review, researching the current primary and secondary literature on the impact of smoking on preeclampsia and especially the changes in retinal vessels occurring related to this.

A first search for articles via PubMed was conducted to get an estimate insight and general information on the topic. The keywords used when first looking for articles were “preeclampsia” and “smoking”. To specify the search other search phrases such as “fundus photography”, “fundus retinoscopy”, “retinal imaging” and “retinal vessels” were added in different combinations.

The search brought up an abundance of articles on preeclampsia, as well as some on the impact of smoking on PE, but only few articles on the change of retinal vessels in relation to the combined main keywords “smoking” and “preeclampsia”

was found this way. Therefore, guidelines were established for the structural search.

First, all articles had to fit the following general criteria to be included in the review

- Articles had to be written in either English or German language
- Articles had to be fully accessible via the Medical University Graz online library admission
- Only articles published after the year 2000 were included

Next the search was split further into three subcategories to define the search results more accurately. For these three subcategories search phrases were established.

The first search phrase contained the combined MESH terms “smoking” and “preeclampsia” brought up 1033 articles. For the second results the MESH term “preeclampsia” was combined with “retinal microvasculature” bringing up 30 results, and for the third search “smoking” and “retinal microvasculature” were used, with 168 results.

Articles were further selected by a screening for their titles and their abstracts subsequently, when selected as suitable the whole article was read before being included in the review.

Additionally, the reference list of reviewed articles was checked to include relevant studies on the topic. Finally, 13 articles were included in the literature review.

The acquired articles were split into the corresponding categories which are as follows each the impact of smoking, pregnancies, and preeclampsia on the retinal vessel as well as the correlation of smoking on preeclampsia.

I chose Zotero as citation program and used it for the management of literature and citations.

In the international scientific literature, there are several definitions of preeclampsia. To clarify the use of the term preeclampsia, I decided to use the definition by the ACOG which also seems to be the most common definition in literature as well as in daily medical practice.

Additional sources such as textbooks, articles and reviews were used to gather information used in the introduction and discussion.

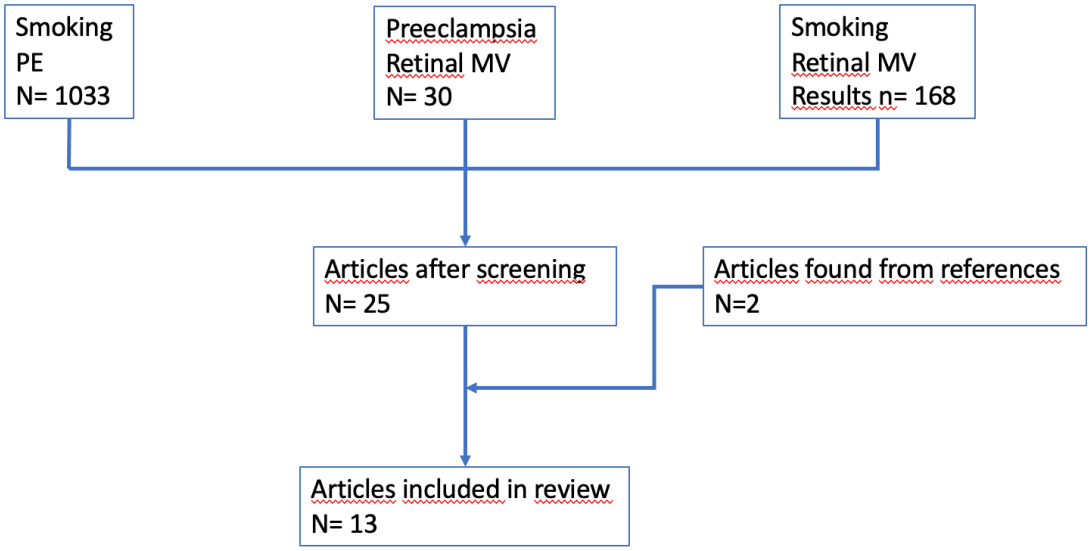

Figure 7: Selection process of studies included in the review

4 Review of current literature

Preeclampsia and smoking have been brought in relation by past studies, but research is still investigating on the topic and no conclusive results have been found so far. The meta-analysis "Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies" by Wei et al., from 2015 took 17 prospective studies from Europe, North America, and Japan. To this point it is the latest meta-study on the topic summarizing the last 20 years of research up to its publication. Their analysis came to the result that there was a significant inverse association between smoking during pregnancy and the incidence of preeclampsia (RR = 0.67, 95% CI 0.60-0.75), with considerable heterogeneity, women smoking ever during pregnancy had a reduced risk of preeclampsia by 33%. (Wei *et al.*, 2015).

Similar results, describing the protective effect of smoking, have been produced by another earlier systematic review from 2007 by England and Zhang. Taking into account 48 epidemiologic studies from 1959 until 2006, it showed a reduction of up to 50% depending on the risk of preeclampsia. (England and Zhang, 2007).

Author	Titel	Type of study	Results
England and Zhang, 2007	Smoking and risk of preeclampsia: a systematic review	systematic review	Review of 48 epidemiological studies brought up a up to 50% reduced risk of PE through smoking. Based on the literature the relationship seems causal
Wei et al., 2015	Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies	systematic review, meta-analysis	Review of 17 with overall 62,098 participants summarized a decreased risk for PE by 33%

Table 2: Reviewed meta-analyses on the association between smoking and PE

Logically this seems paradoxical since smoking is well known to contribute to hypertension and consequential cardiovascular diseases (CVD). The reasons for the possible protective effect of smoking are still debated about. Analysing and comparing the retinal images of smokers, pregnant women, and women with preeclampsia for changes in the microvasculature might help to get some understanding of the interaction of the disease and smoking on the microvasculature.

4.1 Impact of smoking on retinal vessels

Most studies on the effect of smoking on the retinal vessels focus on its diagnostic meaning for CVD, for which smoking is a major risk factor. When comparing the changes to retinal vessels by smoking it is important to correct the values for confounding factors which was done appropriately in all included studies. The

most important of these confounding factors are age, size, diabetes, blood pressure, leukocyte count, BMI, and lipid levels.

Another advisable point when researching on smoking is to check for the classification of the smoking status, since it may differ from article to article in terms of period of smoking and numbers of cigarettes. Since this data is often self-reported by the participants it might be not exact.

Author	Titel	Type of study	Results
Kifley et al., 2007	Long-term Effects of Smoking on Retinal Microvascular Caliber	Population-based cross-sectional and longitudinal study (Blue Mountain Eye study)	Current smokers had a 10.8 µm wider CRVE and a 7.9 µm wider CRAE than non-smokers. Dose-dependent relations between smoking and retinal vessel calibre were found
Ikram et al., 2004	Are Retinal Arteriolar or Venular Diameters Associated with Markers for Cardiovascular Disorders? The Rotterdam Study women with preeclampsia	Population-based cross-sectional study (Rotterdam study)	Smoking is associated with a wider venular diameter, a wider arteriolar diameter and a lower AVR. Smokers had in average a 10 µm wider venular diameter than non-smokers, a 5 µm wider arteriolar diameter and a -0.007 lower AVR
Yanagi et al., 2014	Is the Association Between Smoking and the Retinal Venular Diameter Reversible Following Smoking Cessation?	Retrospective cohort study (Adult Health study of Japanese atomic bomb survivors)	CRVE increased dose-dependent in women who smoked. Women with 1-9 cigarettes/day had a 6.97 µm larger CRVE;10-19 cigarettes/day had a 9.52 µm larger CRVE; ≥20/day cigarettes had a16.9 µm

			larger CRVE
McRae et al., 2019	Inhaled carbon monoxide increases vasodilation in the microvascular circulation	case-control study	After inhalation of CO microvascular reactivity was measured, showing dilatation of the microvasculature

Table 3: Reviewed studies on the impact of smoking on the retinal microvasculature

The retrospective study “Long-term Effects of Smoking on Retinal Microvascular Caliber” by Kifley et al. took data, originally collected by the Blue Mountains Eye study from 1996, which measured the participants retinal vessels twice with a 5-year gap to estimate changes and took detailed information on their smoking behaviours, including time of smoking, number of cigarettes and former smoking behaviour of past smokers. As an outstanding feature this study also adjusted the CRAE and CRVE values to their counterparts, to rule out associations “confounded by shared factors that determine the calibre of both vessels, such as genetic factors or body size” (Kifley *et al.*, 2007). In total the data of 3006 people was selected for analysis, of which 428 were classified as current smokers and 1109 as past smokers.

The results showed significant wider CRVE in current (230 µm) and past smokers (222.6 µm) and a significant wider CRAE in current smokers (196.4 µm), as compared to non-smokers (CRAE 188.3 µm; CRVE 219.2 µm) associating smoking with changes to the retinal venules and possibly to a lesser degree with arterioles. Another result of the study was a significant correlation between smoking and increased leukocyte numbers and the widening of venular vessels, as well as a dose dependent correlation between a wider CRVE and numbers of cigarettes smoked or pack-years of smoking.

The 5-year follow up showed increased odds for changes, both widening and narrowing, in the venular calibre for people in the smoking category group compared to non-smoking participants.

The second inquiry showed that participants who had quit smoking within the last 5 years had wider CRAE and CRVE respectively than participants who had quit smoking for more than 5 years suggesting the correlation to be reversable to some extent.

The study “Are Retinal Arteriolar or Venular Diameters Associated with Markers for Cardiovascular Disorders? The Rotterdam Study” by Ikram et al. put the AVR in context with blood pressure and other cardiovascular risk markers. With a large study group of 5674 participants it showed a relation between smoking and a wider venular diameter and to a smaller degree to a wider arteriolar diameter as well, therefore resulting in a lower AVR. The difference in arteriolar diameters was 5 µm in smokers compared to non-smokers and 1.3 µm in former smokers compared to non-smokers. The greater changes in the venular diameters were 10 µm larger in current smokers than non-smokers and 2.5 µm larger in former smokers compared to non-smokers.
The study showed that wider venular diameter was also correlated to other risk and inflammation markers such as higher leukocyte count, higher erythrocyte sedimentation rate, lower serum HDL levels, higher total serum cholesterol and a higher waist-to-hip ratio, linking it with general systemic signs of inflammation (Ikram *et al.*, 2004).

A Japanese study “Is the Association Between Smoking and the Retinal Venular Diameter Reversible Following Smoking Cessation?” by Yanagi et al. showed similar results in the increase in CRVE value in smokers.
The study is based on a population of atomic bomb survivors, who were under health examination since 1958 and had their smoking behaviour recorded since 1963, it was conducted from 2006 to 2008 and participants were categorized in current smokers, never smokers, non-smokers for more than 10 years, and non-smokers for less than 10 years. Additionally, the numbers of cigarettes/day were recorded as well. The results didn’t show any significant associations between smoking and changes in the retinal microvasculature in men. Women who were smoking also didn’t have significant associations in CRAE but an increased CRVE dependent on the numbers of cigarettes smoked per day. “Females who smoked [up to] 10 cigarettes per day had a 6.9 µm wider mean CRVE (P = 0.001) than nonsmokers” (Yanagi *et al.*, 2014) women who smoked between 10 and 20 cigarettes/day had a 9.52 µm larger CRVE and those who smoked more than 20 cigarettes/day the mean CRVE was 16.9 µm larger respectively.

The study concluded that after 10 years of non-smoking, CRVE was no longer significant wider than never smokers suggesting a reversibility of the effect of smoking to some extend after 5 to 10 years (Yanagi *et al.*, 2014).

The above represented studies are all primarily focused on their association with CVD and had a generally mixed population base, however PE only affects females in a certain age group. Common risk factors for CVDs are an old age and male gender, contrary to the group of young females who are exclusively affected by PE. A study trying to look specifically into PE might be more precise in its proposition, when targeting women in this age group.

Such as the article “Inhaled carbon monoxide increases vasodilation in the microvascular circulation” by McRae et al. from 2019 recruited women over 18 years old without pre-existing medical conditions who did not smoke regularly. The study focuses on the relation between carbon monoxide (CO), which acts as a vasodilator and is produced as a main ingredient of smoke during the combustion of tobacco and the microvasculature, though not specifically the retinal microvasculature. Since the study by Wikström et al. suggested that smokeless tobacco consumption as for example snuff doesn’t reduce the risk for preeclampsia it is assumable that the protective effect of smoking is in fact related to the smoke or its contents (Wikström, Stephansson and Cnattingius, 2010). In their test group McRae et al. administered inhalative low-dose CO (250 ppm for 24 min) to their participants and via laser speckle contrast imaging (LSCI) measured microvascular flux on the forearm during and after occlusion of the artery. The differences in flux can be used to express the microvascular reactivity which can be used as an indirect assessment of vasodilatation. CO treatment showed an increase in the value for microvascular reactivity hypothesized by the authors to be caused by increased vasodilatation. (McRae *et al.*, 2019)

Several other studies doing research on retinal vessel calibres also observed that smoking in general leads to venular widening (Wong *et al.*, 2003; Wimpissinger *et al.*, 2005; Hughes *et al.*, 2009). All the reviewed articles show a common agreement on the widening effect of smoking on the retinal vessels specifically the retinal venules. Arguably this is due to the inhalation of the combustion product

CO which leads to dilatation of the microvasculature but more research which can be expected is needed to further confirm this relation.

4.2 Impact of pregnancy on retinal vessels

Though many articles have been published on physiological changes during pregnancies, the study situation on the effect on the retinal microvasculature is rather sparse. As a woman's body goes through several changes during pregnancy, it would seem likely that the microvasculature is also affected by this and adapting to it. It has been established that the vessels of the mother dilate early during pregnancy but to what degree this is mirrored by the microvasculature of the eye still needs to be researched more deeply.

Author	Titel	Type of study	Results
Lupton et al. 2013	Temporal changes in retinal microvascular caliber and blood pressure during pregnancy	Prospective cohort study	Shows an increase in CRAE and CRVE between 13th and 19th week of gestation, after which it regressed until it met baseline diameter after 6 months postpartum
Lupton et al. 2012	Use of retinal imaging to characterise physiological vascular changes throughout pregnancy	Prospective cohort study	Significant dilatation of arterioles between ±13th and ±19th week of gestation

Table 4: Reviewed studies on the changes to the retinal microvasculature during pregnancy

The study "Use of retinal imaging to characterise physiological vascular changes throughout pregnancy" by Lupton et al. from 2012 was one of the first to measure changes in the retinal microvasculature during healthy pregnancies.

The study took data of 19 healthy women whose retinal images and blood pressures were taken around the 13th, 19th, 29th and 38th week of gestation. CRAE and CRVE were measured with the help of the IVAN software. The collected data showed an increase in CRAE and decrease of diastolic blood pressure between the 13th and 19th week only, the CRVE and systolic blood pressure were not significantly changed (Lupton *et al.*, 2012). These changes during the early part of the pregnancy and their reversion might hint at an adaption phase of the body and the circulatory system.

Another study was performed by the same authors one year later with a bigger sample size, called “Temporal Changes in Retinal Microvascular Caliber and Blood Pressure During Pregnancy”. They collected retinal images of 53 women at 13th, 19th, 29th and 38th weeks of gestation and 6 months postpartum, as well as their corelated mean arterial blood pressure. The study concluded that the retinal microvasculature physiologically changes during the course of a normal pregnancy. Results showed an increase in the CRAE by 6.5 µm (3.9%) and CRVE by 6.6 µm (2.8%) between the 13th and 19th week of gestation, accompanied by a decrease in blood pressure by 4.6mmHg (5.7%), with the changes reverting after the 19th week until delivery (Lupton, Chiu, Hodgson, Tooher, Lujic, *et al.*, 2013). These studies results conducted from a bigger group of participants than the first study further emphasize that changes in the retinal vessels and therefore the microvasculature happen around the 13th to 19th week of gestation.

4.3 Impact of preeclampsia on retinal vessels

PE affects the endothelium of vessels and therefore changes the microvasculature. As mentioned before the retinal microvasculature has similar properties to the placental microvasculature and is therefore well suited for non-invasive in-vivo comparison (Lupton, Chiu, Hodgson, Tooher, Ogle, *et al.*, 2013).

Author	Titel	Type of study	Results
Lupton et al., 2013	Changes in Retinal Microvascular Caliber Precede the Clinical Onset of Preeclampsia	Prospective cohort study	n=92; PE=9 Women with PE had a up to 16.5% reduced cCRAE value and up to 18.1% reduced cCRVE value during pregnancy (peak during 19th week)
Brückmann *et al.*, 2015	Altered retinal flicker response indicates microvascular dysfunction in women with preeclampsia	Prospective observational study	n=84; PE=35 Baseline diameter of retinal vessels of women with PE were lower, although not significantly

Table 5: Reviewed studies on the changes to microvasculature in PE

The study “Changes in Retinal Microvascular Caliber Precede the Clinical Onset of Preeclampsia” by Lupton et al. was the first study found, that used retinal imaging in relation to preeclampsia. It is a prospective cohort study including 92 pregnant women of which nine developed preeclampsia. During their pregnancies retinal images were taken at four certain times and CRAE and CRVE were measured, as well as their blood pressure which was used to correct the values to cCRAE and cCRVE.

The results showed reduced values for arterioles and venules in women with preeclampsia throughout the whole pregnancy, significant changes were observed for cCRAE at 13th, 19th and 38th week of gestation and for cCRVE at 13th and 19th week. This study is one of the first to observe, measure and compare the retinal microvasculature in healthy pregnant women and women with PE. Its weakness is that it is limited by its rather small group size, especially the small size of the group with PE (n=9), and should be repeated in a larger scale to underline the findings (Lupton, Chiu, Hodgson, Tooher, Ogle, *et al.*, 2013).

The study “Altered Retinal Flicker Response Indicates Microvascular Dysfunction in Women With Preeclampsia” focuses on dynamic microvasculature assessment via retinal flicker response, but collected baseline diameters of retinal arterioles and venules of women with preeclampsia, women with normal pregnancy and non-pregnant women for control (Brückmann *et al.*, 2015). These baseline diameters can be compared to those of other studies to get a larger set of data on retinal microvasculature in PE. Though arteriolar and venular baseline diameters were lower in women with PE, than in normal pregnant women and the control group, “baseline diameter […] of the arteriole and venule were not significantly different between groups throughout the study period” (Brückmann *et al.*, 2015).
Since these studies results are not significant and its whole research was focused on a different measurement, they can at most be seen as a trend towards narrowing of retinal microvasculature in PE.

“The degree of maternal cardiovascular dysfunction that precedes the onset of preeclampsia is largely unknown” (Lupton, Chiu, Hodgson, Tooher, Ogle, *et al.*, 2013), the retinal microvasculature might contribute in discovering the process leading there. Research on retinal microvasculature and PE is still rare and should be more focused on in the future.

4.4 The correlation of smoking and preeclampsia

Smoking is known to be a risk factor for cardiovascular health by causing endothelial dysfunction. PE on the other hand is caused by endothelial dysfunction. Therefore, one might expect smoking to contribute to the development of PE, but data shows that the opposite seems to be the case. So far, the most consensual results of past studies on the topic of smoking during pregnancies have concluded that the risk of obtaining preeclampsia is reduced by smoking. To what exact mechanisms this owed is still unsatisfactorily explained but different pathophysiological pathways have been examined to get better insight in the processes.

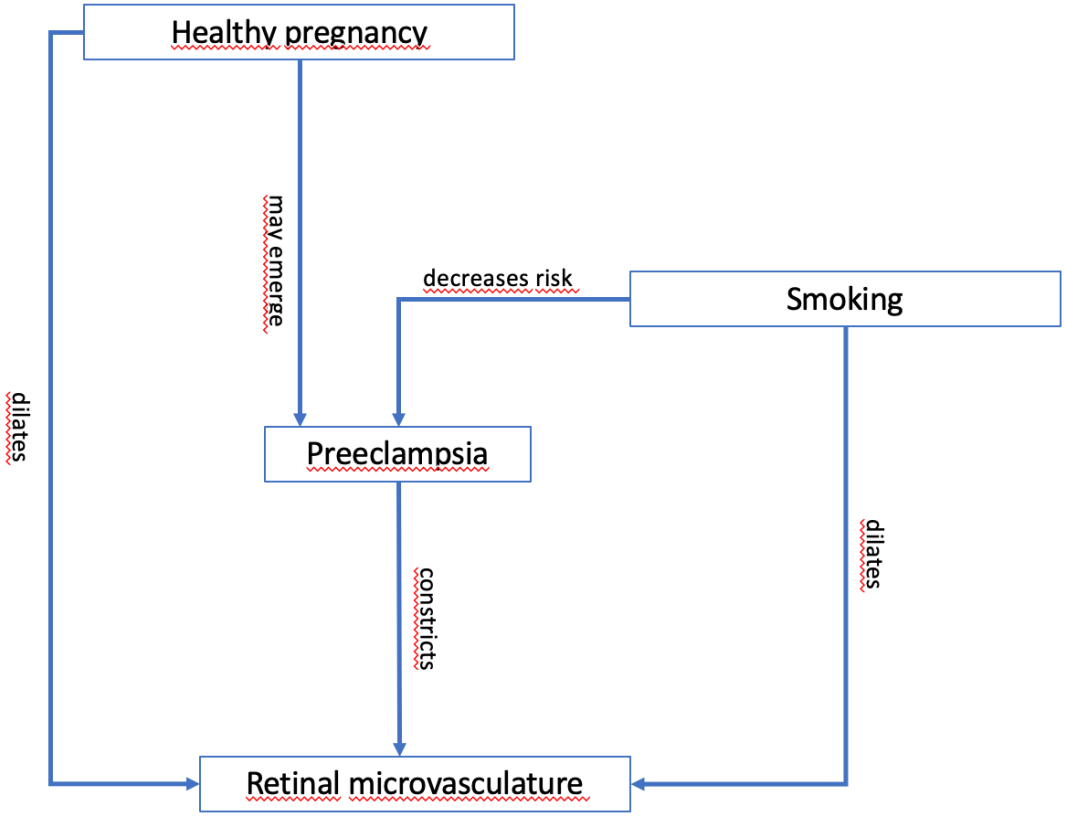

Figure 8: Relationship between the retinal microvasculature, smoking and PE

The above reviewed studies describe the influence of pregnancy, PE, and smoking on the retinal vessels. PE caused a constriction of the retinal microvasculature, while smoking led to a dilatation of the same. These countereffects might partially explain the protective effect of smoking on PE. The following chapter reviews articles that conducted studies, who resulted in a beneficial association between smoking and the risk for PE and investigated on the possible pathophysiological pathways that might be involved in this association.

Author	Titel	Type of study	Results
Wikström et al., 2010	Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff	Retrospective epidemiological study (Swedish Birth Registry), n=605.023	Women who smoked had a dose-dependent decreased risk of PE, in contrary women who used snuff did not

Alpoim et al., 2013	The unexpected beneficial role of smoking in preeclampsia	case-control study, literature review	Women who smoked had a 4.72- fold decreased chance of getting PE
Jeyabalan et al., 2008	Cigarette smoke exposure and angiogenic factors in pregnancy and preeclampsia	case-control study, n=183	Plasma sFLT1 concentrations were lower women who smoked sFLT1/PlGF ratio was lower in women who smoked

Table 6: Studies researching the association between smoking and PE

The study by Wikström et al. “Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff” used the data of the Swedish Birth Registry between the years 1999 and 2006 retrospectively. This birth registry includes near to all births in Sweden, quite thoroughly collects the use of tobacco products around the 15th week of gestation and grades their consumption in these categories: nontobacco user, snuff user, light smoker (< 10 cigarettes/day) and heavy smoker (≥ 10 cigarettes/day). A second study population was formed with women who recorded their use of tobacco products between 30th and 32nd week of gestation to evaluate different outcomes when the habits of tobacco usage changed.

The results were adjusted for confounding factors. They showed that women who smoked had a decreased risk for PE compared to non-smokers (3.01%) dependent on the dose (heavy smokers 1.69%, OR 0.51 (95% CI 0.44-0.58); light smokers 2.32%, OR 0.66 (95% CI 0.61-0.71)) whereas snuff users did even have an increased risk for PE (3.41%, OR 1.11 (95% CI 0.97-1.28)). Interestingly in the second group, women who were registered as smokers on the first survey and then had stopped smoking on the second had a similar risk for PE as women who did not smoke at all with an OR of 0.94 (CI 95% 0.83-1.08). Changes in snuff usage did not seem to alter the risk for PE. The authors concluded that the beneficial effect of smoking might not be due to nicotine, which is contained in snuff products, but rather due to ingredients of cigarette smoke that originate from

combustion of cigarettes, most notably CO. (Wikström, Stephansson and Cnattingius, 2010)

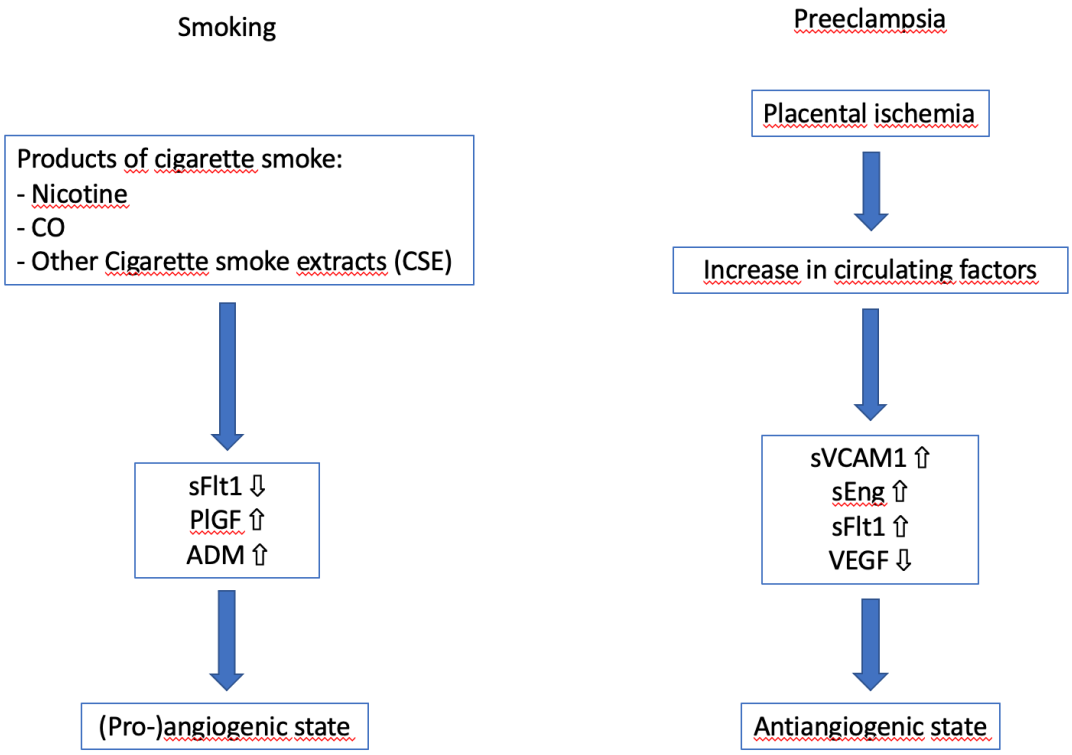

Figure 9: Comparing changes in angiogenic and antiangiogenic factors between smoking and PE

In the article "The unexpected beneficial role of smoking in preeclampsia" from 2016 by Alpoim et al. the authors conducted a small study with 108 preeclamptic women and 104 normotensive pregnancies as control looking into multiple variables such as alleles and phenotypes of different cytokines and levels of coagulation factors and among others smoking. Their results on smoking showed a 4.72-fold decreased chance of getting PE when smoking, coincidently with the previous reviewed studies. The authors summarized several articles researching on factors on a molecular level that might be involved in the reduced risk for PE by smoking (Alpoim *et al.*, 2016).

Multiple angiogenic and antiangiogenic endothelial factors that potentially play a role in the development of PE could be altered by exposition to cigarette smoke. A case-control study by Jeyabalan et al. measured level of sFLT1 and PlGF in four groups of pregnant women in which PE occurred or not and who either smoked or did not. Results showed a significant reduction of sFLT1 in the groups who

smoked compared to the corresponding non-smoking group as well as a trend to an increase in PlGF in healthy smoking group compared to the healthy non-smokers (Jeyabalan *et al.*, 2008). This confirms the findings of an in-vitro study, which found reduced secretion of sFLT1 in term placental villous exposed to cigarette smoke extract (CSE) (Mehendale *et al.*, 2007). Another strong vasodilating peptide and possibly important factor in the development of the placenta is adrenomedullin (ADM) which was increasingly secreted in cytotrophoblast cell lines of animal models afters exposure to CSE (Caron and Smithies, 2002).

These studies show a fundamental insight in the relationship of smoking and PE by establishing hypotheses that might explain the biomechanisms by which smoking during reduces the risk for PE (Alpoim *et al.*, 2016).

5 Discussion

Published articles on the impact of pregnancy and PE on the retinal microvasculature are still scarce but may play an increasingly important role in the future as a non-invasive tool to understand the physiological processes of pregnancy as well as the development of the disease. The impact of smoking on the retinal vessels is supported by a more data but is also more unspecific overall and mostly targets associations to CVD.

There is a continuously growing body of literature on the paradoxically beneficial relation between smoking and PE, but this area of research can still be further explored to get more understanding on the ongoing processes and to establish useful prevention and treatment to the disease.

5.1 Critic and limitations

Although most studies have come to the result that smoking has a protective effect on developing preeclampsia, some have challenged this view. The study “Deconstructing the Smoking-Preeclampsia Paradox through a Counterfactual Framework” by Luque-Fernandez et al. from 2016 claims that these and other

findings are due to methodical errors including selection bias (and others). Using the Icelandic Birth Registry to conduct their own case-control study, they found “the bias-adjusted estimation of the smoking effect on preeclampsia showed an OR of 1.22 (95% CI 0.41-6.53)” (Luque-Fernandez *et al.*, 2016)
Smoking and PE also have some of the adverse pregnancy outcomes in common, such as spontaneous abortion, abruptio placenta or the risk for preterm delivery and reduced birthweight (‘Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy’, 2000; Polańska and Hanke, 2004). The study by Miller et al. 2010 inspected and compared the adverse pregnancy outcomes of smokers and non-smokers, as well as preeclamptic women and women without PE. Women who happened to smoke and additionally got PE had a more than doubled risk on adverse pregnancy outcomes. Their conclusion states that even though smokers were less likely to get PE than non-smokers, the adverse pregnancy outcomes do not justify smoking as protection against PE (Miller *et al.*, 2010)

In need for evaluable studies this literature review also included articles that are somewhat loosely connected to the main topic but still gave some estimation on certain relations that were explored, such as McRae’s study on the effects of CO on the microvasculature and Brückmann’s study measuring retinal flicker response.

5.2 Conclusion

The results of the reviewed literature agree on the protective effect of smoking during pregnancy on PE. This effect is most likely due to the inhalation of cigarette smoke, as it could not be reproduced through the consumption of nicotine products. There seems to be a dose-dependent relation between smoking and PE as heavy smoking increases the protective effect as well as a temporal connection since the risk of women who ceased smoking during their pregnancy returned to the average risk for PE of non-smoking women. (Wikström, Stephansson and Cnattingius, 2010)

In relation to the retinal microvasculature smoking by itself dilates the retinal vessels which is the opposite effect that could be observed by PE which leads to a constriction of the retinal vessels (Ikram *et al.*, 2004; Kifley *et al.*, 2007; Lupton, Chiu, Hodgson, Tooher, Ogle, *et al.*, 2013). These counteracting effects present the protective properties of smoking on the level of microvasculature and reveal the cause of the at first glance paradoxical relationship.

On the molecular level the relaxation and contraction of the microvasculature might be mediated by the release of proangiogenic factors through CSE and antiangiogenic factors through a preeclamptic placenta respectively (Jeyabalan *et al.*, 2008; Alpoim *et al.*, 2016). Additionally, the vasodilating effect of CO may play an important role in the risk reduction (Venditti *et al.*, 2013; McRae *et al.*, 2019).

All the represented hypotheses deserve to be more thoroughly researched in future studies. Upcoming research on the topic could be conducted with modern data evaluation methods like neural networking or advanced bioassays of the placenta.

As far as the practical clinical implementation of the results go several fields of application represent themselves.

On the one hand, the fundus photography enables new opportunities in the prognosis and early detection of PE (Lupton, Chiu, Hodgson, Tooher, Ogle, *et al.*, 2013). Its harmless non-invasive approach can be used in prenatal care to prophylactically assess at risk women. Early detection of signs of PE can lead to early treatment and might prevent the disease from developing to a critical point or even at all.

On the other hand, new therapeutic methods could be developed on the basis of CO administration or even medically intervening in the balance of pro- and antiangiogenic factors.

PE continues to be a threat to pregnant women and should be handled with increasing attention and awareness to gather new means for a cure. Ultimately the same goes for smoking in general and during pregnancy especially to which there is no treatment but efficient precaution which should be strengthened just the same.

Literaturverzeichnis

Abalos, E. *et al.* (2013) ‘Global and regional estimates of preeclampsia and eclampsia: a systematic review’, *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 170(1), pp. 1–7. doi:10.1016/j.ejogrb.2013.05.005.

Alpoim, P.N. *et al.* (2016) ‘The unexpected beneficial role of smoking in preeclampsia’, *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 459, pp. 105–108. doi:10.1016/j.cca.2016.05.030.

Bartsch, E. *et al.* (2016) ‘Clinical risk factors for pre-eclampsia determined in early pregnancy: systematic review and meta-analysis of large cohort studies’, *BMJ (Clinical research ed.)*, 353, p. i1753. doi:10.1136/bmj.i1753.

Brosens, I. and Renaer, M. (1972) ‘On the pathogenesis of placental infarcts in pre-eclampsia’, *The Journal of Obstetrics and Gynaecology of the British Commonwealth*, 79(9), pp. 794–799. doi:10.1111/j.1471-0528.1972.tb12922.x.

Brückmann, A. *et al.* (2015) ‘Altered Retinal Flicker Response Indicates Microvascular Dysfunction in Women With Preeclampsia’, *Hypertension*, 66(4). doi:10.1161/HYPERTENSIONAHA.115.05734.

Caron, K.M. and Smithies, O. (2002) ‘Multiple roles of adrenomedullin revealed by animal models’, *Microscopy Research and Technique*, 57(1), pp. 55–59. doi:10.1002/jemt.10046.

Chapman, A.B. *et al.* (1998) ‘Temporal relationships between hormonal and hemodynamic changes in early human pregnancy’, *Kidney International*, 54(6), pp. 2056–2063. doi:10.1046/j.1523-1755.1998.00217.x.

De Boever, P. *et al.* (2014) ‘Fundus photography as a convenient tool to study microvascular responses to cardiovascular disease risk factors in epidemiological studies’, *Journal of Visualized Experiments: JoVE*, (92), p. e51904. doi:10.3791/51904.

De Silva, D.A. *et al.* (2011) ‘Associations of retinal microvascular signs and intracranial large artery disease’, *Stroke*, 42(3), pp. 812–814. doi:10.1161/STROKEAHA.110.589960.

Duley, L. (2009) ‘The global impact of pre-eclampsia and eclampsia’, *Seminars in Perinatology*, 33(3), pp. 130–137. doi:10.1053/j.semperi.2009.02.010.

England, L. and Zhang, J. (2007) ‘Smoking and risk of preeclampsia: a systematic

review', *Frontiers in Bioscience: A Journal and Virtual Library*, 12, pp. 2471–2483. doi:10.2741/2248.
Esplin, M.S. *et al.* (2001) 'Paternal and maternal components of the predisposition to preeclampsia', *The New England Journal of Medicine*, 344(12), pp. 867–872. doi:10.1056/NEJM200103223441201.
Funk, R.H. (1997) 'Blood supply of the retina', *Ophthalmic Research*, 29(5), pp. 320–325. doi:10.1159/000268030.
Geary, M. (1997) 'The HELLP syndrome', *British Journal of Obstetrics and Gynaecology*, 104(8), pp. 887–891. doi:10.1111/j.1471-0528.1997.tb14346.x.
Germain, S.J. *et al.* (2007) 'Systemic inflammatory priming in normal pregnancy and preeclampsia: the role of circulating syncytiotrophoblast microparticles', *Journal of Immunology (Baltimore, Md.: 1950)*, 178(9), pp. 5949–5956. doi:10.4049/jimmunol.178.9.5949.
'Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin Summary, Number 222' (2020) *Obstetrics and Gynecology*, 135(6), pp. 1492–1495. doi:10.1097/AOG.0000000000003892.
Haram, K., Svendsen, E. and Abildgaard, U. (2009) 'The HELLP syndrome: clinical issues and management. A Review', *BMC pregnancy and childbirth*, 9, p. 8. doi:10.1186/1471-2393-9-8.
Hartley, J.D.R., Ferguson, B.J. and Moffett, A. (2015) 'The role of shed placental DNA in the systemic inflammatory syndrome of preeclampsia', *American Journal of Obstetrics and Gynecology*, 213(3), pp. 268–277. doi:10.1016/j.ajog.2015.03.026.
Hemminki, V. *et al.* (2007) 'Determination of retinal blood vessel diameters and arteriovenous ratios in systemic hypertension: comparison of different calculation formulae', *Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie*, 245(1), pp. 8–17. doi:10.1007/s00417-006-0358-7.
Hughes, A.D. *et al.* (2009) 'Determinants of retinal microvascular architecture in normal subjects', *Microcirculation (New York, N.Y.: 1994)*, 16(2), pp. 159–166. doi:10.1080/10739680802353868.
Ichikawi, I. and Harris, R.C. (1991) 'Angiotensin actions in the kidney: renewed insight into the old hormone', *Kidney International*, 40(4), pp. 583–596. doi:10.1038/ki.1991.249.

Ikram, M.K. *et al.* (2004) ‘Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study’, *Investigative Ophthalmology & Visual Science*, 45(7), pp. 2129–2134. doi:10.1167/iovs.03-1390.

Jeyabalan, A. *et al.* (2008) ‘Cigarette Smoke Exposure and Angiogenic Factors in Pregnancy and Preeclampsia’, *American Journal of Hypertension*, 21(8), pp. 943–947. doi:10.1038/ajh.2008.219.

Khan, K.S. *et al.* (2006) ‘WHO analysis of causes of maternal death: a systematic review’, *The Lancet*, 367(9516), pp. 1066–1074. doi:10.1016/S0140-6736(06)68397-9.

Kifley, A. *et al.* (2007) ‘Long-term effects of smoking on retinal microvascular caliber’, *American Journal of Epidemiology*, 166(11), pp. 1288–1297. doi:10.1093/aje/kwm255.

Knudtson, M.D. *et al.* (2003) ‘Revised formulas for summarizing retinal vessel diameters’, *Current Eye Research*, 27(3), pp. 143–149. doi:10.1076/ceyr.27.3.143.16049.

Lachmeijer, A.M.A. *et al.* (2002) ‘Searching for preeclampsia genes: the current position’, *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, 105(2), pp. 94–113. doi:10.1016/s0301-2115(02)00208-7.

Lange, S. *et al.* (2018) ‘National, regional, and global prevalence of smoking during pregnancy in the general population: a systematic review and meta-analysis’, *The Lancet. Global Health*, 6(7), pp. e769–e776. doi:10.1016/S2214-109X(18)30223-7.

Levine, R.J. *et al.* (2004) ‘Circulating angiogenic factors and the risk of preeclampsia’, *The New England Journal of Medicine*, 350(7), pp. 672–683. doi:10.1056/NEJMoa031884.

Lupton, S.J. *et al.* (2012) ‘OS010. Use of retinal imaging to characterise physiological vascularchanges throughout pregnancy’, *Pregnancy Hypertension*, 2(3), pp. 179–180. doi:10.1016/j.preghy.2012.04.011.

Lupton, S.J., Chiu, C.L., Hodgson, L.A.B., Tooher, J., Ogle, R., *et al.* (2013) ‘Changes in retinal microvascular caliber precede the clinical onset of preeclampsia’, *Hypertension (Dallas, Tex.: 1979)*, 62(5), pp. 899–904. doi:10.1161/HYPERTENSIONAHA.113.01890.

Lupton, S.J., Chiu, C.L., Hodgson, L.A.B., Tooher, J., Lujic, S., *et al.* (2013)

‘Temporal changes in retinal microvascular caliber and blood pressure during pregnancy’, *Hypertension (Dallas, Tex.: 1979)*, 61(4), pp. 880–885. doi:10.1161/HYPERTENSIONAHA.111.00698.
Luque-Fernandez, M.A. *et al.* (2016) ‘Deconstructing the smoking-preeclampsia paradox through a counterfactual framework’, *European Journal of Epidemiology*, 31(6), pp. 613–623. doi:10.1007/s10654-016-0139-5.
Marzioni, D. *et al.* (2004) ‘Restricted innervation of uterus and placenta during pregnancy: evidence for a role of the repelling signal Semaphorin 3A’, *Developmental Dynamics: An Official Publication of the American Association of Anatomists*, 231(4), pp. 839–848. doi:10.1002/dvdy.20178.
McRae, K.E. *et al.* (2019) ‘Inhaled carbon monoxide increases vasodilation in the microvascular circulation’, *Microvascular Research*, 123, pp. 92–98. doi:10.1016/j.mvr.2019.01.004.
Mehendale, R. *et al.* (2007) ‘Placental angiogenesis markers sFlt-1 and PlGF: response to cigarette smoke’, *American Journal of Obstetrics and Gynecology*, 197(4), p. 363.e1-363.e5. doi:10.1016/j.ajog.2007.06.025.
Miller, E.C. *et al.* (2010) ‘The risk of adverse pregnancy outcomes is increased in preeclamptic women who smoke compared with nonpreeclamptic women who do not smoke’, *American Journal of Obstetrics and Gynecology*, 203(4), p. 334.e1–8. doi:10.1016/j.ajog.2010.05.020.
Palei, A.C. *et al.* (2013) ‘Pathophysiology of hypertension in pre-eclampsia: a lesson in integrative physiology’, *Acta Physiologica (Oxford, England)*, 208(3), pp. 224–233. doi:10.1111/apha.12106.
Polańska, K. and Hanke, W. (2004) ‘[Effect of smoking during pregnancy on maternal condition and birth outcome--overview of epidemiologic studies]’, *Przeglad Epidemiologiczny*, 58(4), pp. 683–691.
Powe, C.E., Levine, R.J. and Karumanchi, S.A. (2011) ‘Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease’, *Circulation*, 123(24), pp. 2856–2869. doi:10.1161/CIRCULATIONAHA.109.853127.
Rajakumar, A. *et al.* (2012) ‘Transcriptionally active syncytial aggregates in the maternal circulation may contribute to circulating soluble fms-like tyrosine kinase 1 in preeclampsia’, *Hypertension (Dallas, Tex.: 1979)*, 59(2), pp. 256–264. doi:10.1161/HYPERTENSIONAHA.111.182170.

Rana, S. *et al.* (2019) ‘Preeclampsia: Pathophysiology, Challenges, and Perspectives’, *Circulation Research*, 124(7), pp. 1094–1112. doi:10.1161/CIRCRESAHA.118.313276.
Redman, C.W. and Sargent, I.L. (2005) ‘Latest advances in understanding preeclampsia’, *Science (New York, N.Y.)*, 308(5728), pp. 1592–1594. doi:10.1126/science.1111726.
‘Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy’ (2000) *American Journal of Obstetrics and Gynecology*, 183(1), pp. S1–S22.
Rios, D.R.A. *et al.* (2016) ‘Increased Levels of sENG and sVCAM-1 and Decreased Levels of VEGF in Severe Preeclampsia’, *American Journal of Hypertension*, 29(11), pp. 1307–1310. doi:10.1093/ajh/hpv170.
Roberts, J.M. *et al.* (1989) ‘Preeclampsia: an endothelial cell disorder’, *American Journal of Obstetrics and Gynecology*, 161(5), pp. 1200–1204. doi:10.1016/0002-9378(89)90665-0.
Robillard, P.-Y. *et al.* (2019) ‘High incidence of early onset preeclampsia is probably the rule and not the exception worldwide. 20th anniversary of the reunion workshop. A summary’, *Journal of Reproductive Immunology*, 133, pp. 30–36. doi:10.1016/j.jri.2019.05.003.
Romero, R. and Chaiworapongsa, T. (2013) ‘Preeclampsia: a link between trophoblast dysregulation and an antiangiogenic state’, *The Journal of Clinical Investigation*, 123(7), pp. 2775–2777. doi:10.1172/JCI70431.
Ross, K.M. *et al.* (2019) ‘Socioeconomic Status, Preeclampsia Risk and Gestational Length in Black and White Women’, *Journal of Racial and Ethnic Health Disparities*, 6(6), pp. 1182–1191. doi:10.1007/s40615-019-00619-3.
Sanders, J.S., Mark, A.L. and Ferguson, D.W. (1989) ‘Importance of aortic baroreflex in regulation of sympathetic responses during hypotension. Evidence from direct sympathetic nerve recordings in humans’, *Circulation*, 79(1), pp. 83–92. doi:10.1161/01.cir.79.1.83.
Serre, K. and Sasongko, M.B. (2012) ‘Modifiable lifestyle and environmental risk factors affecting the retinal microcirculation’, *Microcirculation (New York, N.Y.: 1994)*, 19(1), pp. 29–36. doi:10.1111/j.1549-8719.2011.00121.x.
Shahoud, J.S., Sanvictores, T. and Aeddula, N.R. (2022) ‘Physiology, Arterial Pressure Regulation’, in *StatPearls*. Treasure Island (FL): StatPearls Publishing.

Available at: http://www.ncbi.nlm.nih.gov/books/NBK538509/ (Accessed: 11 April 2022).
Steegers, E.A.P. *et al.* (2010) 'Pre-eclampsia', *Lancet (London, England)*, 376(9741), pp. 631–644. doi:10.1016/S0140-6736(10)60279-6.
Stevens, W. *et al.* (2017) 'Short-term costs of preeclampsia to the United States health care system', *American Journal of Obstetrics and Gynecology*, 217(3), pp. 237-248.e16. doi:10.1016/j.ajog.2017.04.032.
Sun, C. *et al.* (2009) 'Retinal vascular caliber: systemic, environmental, and genetic associations', *Survey of Ophthalmology*, 54(1), pp. 74–95. doi:10.1016/j.survophthal.2008.10.003.
Venditti, C.C. *et al.* (2013) 'Chronic carbon monoxide inhalation during pregnancy augments uterine artery blood flow and uteroplacental vascular growth in mice', *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 305(8), pp. R939–R948. doi:10.1152/ajpregu.00204.2013.
Venkatesha, S. *et al.* (2006) 'Soluble endoglin contributes to the pathogenesis of preeclampsia', *Nature Medicine*, 12(6), pp. 642–649. doi:10.1038/nm1429.
Wallis, A.B. *et al.* (2008) 'Secular trends in the rates of preeclampsia, eclampsia, and gestational hypertension, United States, 1987-2004', *American Journal of Hypertension*, 21(5), pp. 521–526. doi:10.1038/ajh.2008.20.
Wei, J. *et al.* (2015) 'Cigarette smoking during pregnancy and preeclampsia risk: a systematic review and meta-analysis of prospective studies', *Oncotarget*, 6(41), pp. 43667–43678. doi:10.18632/oncotarget.6190.
Wikström, A.-K., Stephansson, O. and Cnattingius, S. (2010) 'Tobacco use during pregnancy and preeclampsia risk: effects of cigarette smoking and snuff', *Hypertension (Dallas, Tex.: 1979)*, 55(5), pp. 1254–1259. doi:10.1161/HYPERTENSIONAHA.109.147082.
Wimpissinger, B. *et al.* (2005) 'Response of retinal blood flow to systemic hyperoxia in smokers and nonsmokers', *Graefe's Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie*, 243(7), pp. 646–652. doi:10.1007/s00417-004-1083-8.
Wong, T.Y. *et al.* (2003) 'Retinal vessel diameters and their associations with age and blood pressure', *Investigative Ophthalmology & Visual Science*, 44(11), pp. 4644–4650. doi:10.1167/iovs.03-0079.

Yanagi, M. *et al.* (2014) ‘Is the association between smoking and the retinal venular diameter reversible following smoking cessation?’, *Investigative Ophthalmology & Visual Science*, 55(1), pp. 405–411. doi:10.1167/iovs.13-12512.
Zhou, Y., Damsky, C.H. and Fisher, S.J. (1997) ‘Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome?’, *The Journal of Clinical Investigation*, 99(9), pp. 2152–2164. doi:10.1172/JCI119388.