ISSN: 0975-833X

Availableonlineathttp://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 05, pp.3604-3607, May, 2019

DOI: https://doi.org/10.24941/ijcr.35408.05.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

DETECTION OF RISK OF PREECLAMPSIA IN PREGNANCY USING THE UTERINE ARTERY DOPPLER AND CYTOKINES

***Maja Pejkovska Ilieva and Vesna Antovska**

PHI University Clinic for Gynecology and Obstetrics, Faculty of Medicine, Skopje, Republic of Northern Macedonia

ARTICLEINFO

Article History:
Received 28th February, 2019
Received in revised form
04th March, 2019
Accepted 20th April, 2019
Published online 30th May, 2019

Key Words:
Preeclampsia, prediction, cytokines, notch of uterine artery.

**Corresponding author:*
Maja Pejkovska Ilieva

ABSTRACT

Introduction: Preeclampsia is the main cause of maternal and fetal morbidity and mortality in the world. Its etiopathogenesis is multifactorial and is still a challenge and subject of research. **Purpose:** To predict the occurrence of preeclampsia in patients in the second trimester of pregnancy who are asymptomatic with the help of immunological biomarkers. **Materials and Methods:** Total of 100 patients is being examined and is divided in two groups. An examined group of 50 patients was with notch of the uterine artery and the interaction of the more pro-inflammatory with anti-inflammatory cytokines (TNF-α, IL-1α, IL-2, IL- 6 versus IL-4, IL-10). The ELISA (Enzyme-linked immunosorbent assay) methodology is used. Also a control group of 50 patients without uterine artery notch is examined for comparison and drawing appropriate conclusions. **Results and Discussion:** The obtained results of increased proinflammatory and reduced regulatory anti-inflammatory immune cells and cytokines, creates an uncontrolled state of inflammation. The rise in IL6 together with TNF-α with a significance of $p < 0.01$ and the decrease in mean in anti-inflammatory cytokines (IL4 and IL10) compared to the control group with the investigated group are important and credible parameters for the expected further development of the preeclampsia state. This disorder is thought to contribute to the overall preeclampsia pathophysiology. **Conclusion:** Uterine Doppler together with immunological biomarkers increases the sensitivity and specificity of diagnostics of the clinical preeclampsia syndrome.

Copyright©2019, Maja Pejkovska Ilieva and Vesna Antovska. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

Citation: Maja Pejkovska Ilieva and Vesna Antovska. **2019.** "Detection of risk of preeclampsia in pregnancy using the uterine artery doppler and cytokines", *International Journal of Current Research*, 11, (05), 3604-3607.

INTRODUCTION

Preeclampsia is a multisystem disorder occurring in the range of 5 to 10% of pregnancies (Francisco J. Valenzuela *et al*., 2011). It represents a threat to the health of pregnant women during and after termination of pregnancy, but it is also a threat to the condition of the fetus intrauterine, consequential and postpartum (Tessa E.R. Gillon *et al*., 2014). Preeclampsia according to the American College of Obstetricians and Gynecologists is defined as the presence of blood pressure greater than or equal to 140/90 mmHg after the 20th gestation week. There is presence of proteinuria, thrombocytopenia and elevated hepatic transaminases associated with this condition (ACOG, 2013; Kathleen A. Pennington *et al*., 2012). There is systemic dysfunction of the endothelium and microangiopathy, at the level of the kidney, leading to glomerular endotheliosis, even occurrence of acute renal failure. In the lungs, pulmonary edema can be developing. At the level of the brain, occurrence of eclampsic attacks and eclampsia, visual disturbances, cerebral haemorrhage can appear. HELLP syndrome, DIC, that is, life-threatening conditions and eventual death can happen in the worst cases (Arvind Goel and Saroch Rona, 2014). The relationship between immunology, inflammation and preeclampsia is shown in pregnancy, by adaptation of the immune response needed for the mother to tolerate the different immune system from the fetus (Leukoc Biol, 2013). The placenta creates an entire collection of pro and anti-inflammatory cytokines, adipocins, and cytokine-like angiogenic growth factors that, with pre-eclampsia due to the occurrence of hypoxia, have altered ratios (Madhur Gupta and Suresh Chari, 2015; Govana Ogge *et al*., 2011). SEPS 1 is an inflammatory responsible gene that is responsible for the elevated values of proinflammatory cytokines and other mediators of inflammation in preeclampsia (Francisco J. Valenzuela *et al*., 2011). Immunological adaptation to normal pregnancy is associated with a reduction in T helper 1 cytokines interleukin2 (IL2), interferon gamma (IF γ), transforming growth factor (TGF β), where the cellular immunity is involved and thus they mediate and inhibit the immunological non-acceptance of the fetus. At the same time, there is an increase in T helper cytokines, IL4, IL5, IL6, and IL13 that mediate humoral immunity, suppress cell immunity, and thereby prevent the immunological rejection of the fetus (Ifeoma Udenze *et al*., 2015). The abnormality in this immunologically balanced response is the responsible cause in the pathology of the preeclampsia. The failure of trophoblastic

invasion results in reduced blood flow to the placenta and fetus and consequently by placental ischemia (Casey Berry and Mohamed G Atta, 2016). The ischemic placenta in turn indicates the release of bioactive circulating factors including proinflammatory cytokines that contribute to the mediation of the wide endothelial damage zone pathognomatically for preeclampsia. This immunological imbalance contributes to the overall pathophysiology of preeclampsia. There is a production of the so called reactive oxygen spec. (ROS), increased endothelin 1 (En1), and Beta-cell autoantibody production, such as angiotensin II (Ang II) type 1 receptor (AT1 -AA), which in general culminate the development of hypertension in pregnancy (Alicia Martinez-Varea *et al*., 2014). The imbalance itself worsens as pregnancy progresses. Proinflammatory cytokines secreted from the activated Th1 and Th17 cells promote cytotoxic and inflammatory response (Laresgoiti-Servitje *et al*., 2010). In the vascular basin, elevated levels of TNF α (tumor necrosis factor alpha) and IL6 (interleukin 6) both contribute to endothelial dysfunction predisposing preeclampsia and is characterized by increased adhesion of the molecules and endothelial cell permeability (Babbette La Marca, 2013).

Purpose

The purpose of this article is to examine and verify the effect of de novo repopulation of immunomodulators as regulation of the immune response in the pathophysiology of preeclampsia. The aim is to predict the occurrence of preeclampsia in patients in the second trimester of pregnancy who are asymptomatic. As an initial test criterion, an ultrasound observed notch of arterial uterine was detected and then the immunomodulators value was added in order to obtain a more predictive or prognostic value in the second trimester to determine the possible risk of preeclampsia.

MATERIAL AND METHODS

Examinations were performed at the PHI University Clinic for Gynecology and Obstetrics – Skopje, Republic of Northern Macedonia. Namely, 100 patients were examined, divided into 2 groups: investigated and control. The investigated group consisted of 50 patients in the second trimester, besides the routine ultrasonographic examination for monitoring fetal growth and development, also had ultrasonographic Doppler for the uterine artery performed. The presence of a notch of uterine artery (an abnormal waveform of the uterine artery flow due to a high resistance index results in the presence of an early diastolic node) is the main inclusion criterion of the examined group. These patients are suspected of developing preeclampsia. For the timely diagnosis of preeclampsia, those patients are analyzed for the circulating immunological biomarkers in the patient's serum and importantly the levels of cytokines are examined. The deviations in production and the interaction of proinflammatory with antiinflammatory cytokines (TNF-α, IL-1α, IL-2, IL-6 versus IL-4, IL-10) are more specifically investigated. They are made using the ELISA (Enzyme-linked immunosorbent assay) method. It is prepared at the Institute of Immunology and Human Genetics at the Medical Faculty in Skopje with a special kit - Magnetic Human Luminex Assay. The control group consists of 50 pregnant patients in the same gestational age in the absence of notch of uterine artery, without hypertension that has also been studied in these institutions in order not to overlook the non-manifest hidden preeclampsia. Exclusive criteria of the investigated and control group include patients with pre-existent hypertension,other immunological and haematological diseases. A detailed anamnestic data on pregnancy were taken before and during the examination. The patients have signed an informed consent to participate in the study. During the ultrasonographic examination, after a detailed examination of the fetus, special attention is paid to the flow of uterine artery. It is performed on ultrasonographic apparatus Voluson 8 (General electronics) with transabdominal probe 3-5 MHz. Doppler testing is performed by standard technique. Patients with a positive result are sent to the Institute of Immunology and Human Genetics and from the serum where the values of cytokines are verified wth ELISA (TNF-α, IL-1α, IL-2, IL-6, IL- 4, IL-10). The results obtained are subject to statistical analysis and are being processed with the program STATISTICA 7 and SPSS 17.0 for Windows. A T-test for the significance of differences between the comparing groups of all the analyzed parameters was made. Significance and significant differences are present in all analyzed parameters with a dimension of $p < 0.001$ to $p < 0.005$. Contrary to normal pregnancy, preeclampsia has an inadequate immune response and a range of alternatively activated immune cells and cytokines.

RESULTS

Based on the results obtained from the follow up, it can be concluded that the combination of the ultrasoundly verified notch of the uterine artery and the subsequent changes in the cytokine values signalize the preeclampsia in the second trimester. In the course of the investigation, 4 patients prematurely terminated pregnancy due to premature rupture of the membranes before the 22-nd gestation week. With this, the investigation was realized in both groups of 48 patients or 96 in total. The results for all immunomodulators are monitored individually, their correlation, the results of significance, the T test for significance of differences and the comparison in the variations between the mean values of the two examined groups. From the obtained results, there is a visible increase in IL-6 values at the level of correlation between the variables. Significance was obtained in 99% with value of the $p < 0.01$. Furthermore, proinflammatory cytokines TNF-α grows along with IL-6, which is significant with value of the $p < 0.05$. When IL-1α / I-1F1 grows, the value of IL-2 with a significance of $p < 0.05$ increases. In comparison, antinflammatory cytokines follow a tendency to decline i.e. IL-4 in the control group is 2.78 pg / ml and in the investigated group is 1.55. The same and IL-10 in the control group is 0.96, and the investigated group is 0.14, which means that there is a tendency of decreasing. The analytical results of the tests performed in total synthesized volume are shown in Table 1 and are detailed in Tables 2. The analysis of the control group IL-1α / I-1F1 are statistically significant. Along with it grows IL-2 and not statistically, but insignificantly decreases IL-6 which is within the range of a normal pregnancy where we have control of proinflammatory cytokines and limitation in their growth. In contrast, the IL-6 in the examined group grows and along with TNF-α together, with a mutual tendency to grow, which is statistically significant with the value of the $p < 0.01$.

DISCUSSION

Preeclampsia is associated with hypertension after 20 weeks of gestation, reduced renal function, small for gestational age (SGA) fetuses, etc. (Pilalis *et al*., 2007). In healthy pregnancy,

the decreased Th1/Th2 ratio in addition to higher values of Th2 cells in the peripheral circulation of the mother maintains the immunotolerance to the fetus. Balance is the most important and derived circulatory cytokines derive from it, proinflammatory and anti-inflammatory. T regulatory cells (Treg) are essential for normal pregnancy, due to the created immunotolerance with regulation of Th 1 by T naive cells and at the same time their ability to produce IL10 (Jonsson *et al*., 2004). With the decrease in Th1 cells, the proinflammatory cytokines responsible for inflammation are diminished. IL10 itself induces expression of the transcription factor expression (Foxp3), which is inevitable for the production of Treg as a recovery back up maintenance mechanism (Babbette La Marca, 2013). TNF-α (tumor necrosis factor alpha) has a major role in the cytokine network of a wide range of bioactivity. It is normally produced by placental trophoblast cells and fetoplacental macrophages (Xie *et al*., 2014). So its role is to activate endothelial cells, increase coagulation, increase expression of adhesion molecules, increase vascular permeability and induce microvascular leakage. It activates the endothelin system in the kidney (Madhur Gupta and Suresh Chari, 2015; Lamarca, 2012). IL6 proinflammatory cytokine, produced by mononuclear phagocytes, endothelial cells, fibroblasts, and T cells is involved in immunological activation, function in vascularization, and modulation of TNF-α production. It increases the permeability of endothelial cells by changing the shape of the cells and by reorganizing the intracellular actin fibers (Babbette La Marca, 2013; Jones, 2005).

It is involved in neutrophil activation, in the expression of Von Willebrand, and in the cellular adhesion of endothelium due to vascular damage. It activates the rennin angiotensin system with a result of vasoconstriction (Jonsson *et al*., 1998). It is with the results presented for its significant growth that proves that proinflammatory cytokines are uncontrolled and are in growth in the preeclampsia syndrome. IL1 increases the production of thrombin and coagulation. It induces production of platelet activating factor (Taylor *et al*., 2016). IL2 participates in the regulation of the expression of decidual cells. IL4 anti-inflammatory cytokine inhibits IL2 and induced increased cytotoxicity. It makes controlled proliferation of epithelial cells. It inhibits the production of IL1, and IL6 TNF-α using monocytes (András Szarka *et al*., 2010). All these facts have been proven in this examination as well. In preeclampsia, decreased levels of IL10 are monitored in both the circulation and placental level. IL10 itself has the ability to reduce inflammatory cytokines associated with oxidative stress in order to have appropriate remodeling of the spiral arteries and sufficient placental perfusion. It has also been explored as a therapeutic method that reduces endothelin 1 and IFNγ, restores vasodilatation, and reduces proteinuria. The very reduction in T regulatory cells and their correlation with regulatory cytokine IL10 has been proven to be associated with placental ischemia in pregnancy. It is a challenge and a premise of research of numerous experts in this field. Anti-inflammatory cytokines that help regulate the immune response, such as interleukin IL 10 and IL4 play a key role in normal and successful pregnancy ensuring the balance of the immune system. As a result of this study is the effect derived from the immunological therapy, that is, with therapeutic protocols of adding T regulatory cells or the transfer of IL10 to restore the balance of the immune system in order to reduce the elevated blood pressure. So, in mutual, the immune cells in pregnancy caused by the decidua have the role of providing adequate implantation and promoting a trophoblastic invasion, which is neither superficial nor too invasive. This is achieved by the production of cytokines and oncogenic factors, which is necessary for normal pregnancy.

Conclusion

From the investigation and obtained results, adjuvant methods in diagnosing pregnant women with preeclampsia will be enabled. That will mean verification of the immunological component in the etiopathogenesis in preeclampsia as a justification in diagnosing this syndrome. Early diagnosis and treatment of preeclampsia is of vital, crucial significance for maintenance of the life and health of the mother and of the baby. With modern possibilities for establishing proper education of the gynecologist-obsteretition, it is allowed early discovering, diagnosing and complete treatment in the purpose of prevention from the negative consequences that can occur. Patients need to be monitored postpartum as well for prevention of appearance of this pathological state in the next pregnancies.

REFERENCES

ACOG, 2013. American College of Obstetricians and Gynecologists Task Force on Hypertension in Pregnancy, issuing body. Title (DNLM: Hypertension, Pregnacy-Introduced – Pracice Guideline. WQ 244). RG575.5 ISBN 978-1-934984-28-4.

András Szarka, János Rigó, Jr, Levente Lázár, Gabriella Bekő, and Attila Molvarec Circulating Cytokines, 2010. Chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array PMCID: PMC3014878.

Arvind Goel and Saroch Rona, 2014. Angiogenic factors in preeclampsia: potential for diagnosis and treatment. Curr Opin Nephrol Hypertens. Author manuscript PMCMay 1 Publeshed as: *Curr Nephrol Hypertens.,* 2013 Nov(6) doi PMCID: PMC3932709.

Babbette La Marca, 2013. The role of immune activation in contributing to vascular dysfunction and the pathophysiology of hypertension during preeclampsia. Minerva Ginecol. Author manuscript; Publeshed in final edited form as: Minerva Ginecol (2) 105-120 PMCID: PMC3740963.

Casey Berry and Mohamed G Atta. 2016. Hypertensive disorders in pregnancy. *World J. Nephrol*., 2016 Sep 6 PMCID:PMC5011248.

Francisco J. Valenzuela, Alejandra Perez-Sepulveda, Maria J. Torres, Paula Correa, Gabriela M. Repetto and Sebastian E. Illanes. 2012. Pathogenesis of preeclampsia: *The Genetic Component. J Pregnancy*; Published online 2011 Dec 1 doi: 10.1155/2012/632732 PMCID: PMC3235819

Francisco J. Valenzuela, Alejandra Perez-Sepulveda, Marıa J. Torres, Paula Correa, Gabriela M. Repetto and Sebastian E. Illanes, 2011. Pathogenesis of Preeclampsia: The Genetic Component,

Govana Ogge, Tinnanorn Chaiworapongsa, Roberto Romero, Yousset Hussein, Juan Pedro Rusanovic, Lami Yeo, Chong Jai Kim and Sonia S. Hassan, 2011. Placental lesions associated with maternal underperfusion are more frequent in early–onset than in late-onset preeclampsia. *J. Perinatal Med.,* Author manuscript doi 10.1515/JPM 2011.098 PMCID: PMC3213694.

Ifeoma Udenze, Casimic Amadi, Nicolas Awolola,Christian chigozie Makwe, 2015. The role of cytokines as inflammatory mediators in preeclampsia. Pan Afr Med J.2015 20:219 doi 10.11604/pamj2015.20.219.5317 PMCID: PMC4470443.

Jones, SA. 2005. Directing transition from innate to acquired immunity: defining a role for IL-6. *J Immunol.,* 175:3463–3468. [PubMed]

Jonsson, Y., Ekerfelt, C., Olsson, H., Boij, K., Berg, G., Ernerndh, J. and Metthiesen, L. 1998. Immunohisto chemical studies of interleukin – 4(IL-4) and tumor necrosis factor –l- (TNF- l) in placentas from preeclamptic and normal pregnancies. Manuscript.

Jonsson, Y., Ekerfett, C., Berg, G., Nieminen, K., Sharma, S., Ernerudh, J. and Matthiesen, L. 2004. Systemic Th1/Th2 cytokine responses to paternal and vaccination antigens in preeclampsia no differences compared with normal pregnancy. *Am J Reprod Immun.,* 51 (4):302-310.

Kathleen A. Pennington, Jessica M. Schlitt, Daniel L.Jackson, Laura C.Schultz and Danny J. Schist, 2012. Preeclampsia: Multiple approaches for a multifactorial disease. Dis Model Mech 2012 Jan 5 (9-18)doi 10.1242/dmm.008516 PMCID : PMC3255538.

Lamarca, B1. 2012. Endothelial dysfunction. An important mediator in the pathophysiology of hypertension during pre-eclampsia Minerva Ginecol., 64(4):309-20. PMID: 22728575 PMCID: PMC3796355.

Laresgoiti-Servitje, E., Gómez-López, N. and Olson, DM. April 2010. "An immunological insight into the origins of pre-eclampsia". Hum Reprod Update. 16 (5): 510–24. doi:10.1093/humupd/dmq007. PMID 20388637.

Leukoc Biol, J. 2013. A leading role for the immune system in the pathophysiology of preeclampsia, Epub 2013 Apr 30 doi 10.1189/jlb.112603 PMID: 23633414.

Madhur Gupta and Suresh Chari, 2015. Predictive value of inflammatory Cytokines in preeclampsia, International Journal of Biomedical and Advance Research 2015, ISSN: 2229-3809 (Online), Journal DOI: 10.7439/ijbar, CODEN: IJBABN.

Pilalis, A., Souka, A. P., Antsaklis, P., Daskalakis, G., Papantoniou, N., Mesogitis, S. and Antsaklis, A. 2007. Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11–14 weeks' gestation. *Ultrasound Obstet Gynecol,* 29: 135–140. doi:10.1002/uog.3881

Taylor, BD[1], Ness, RB[2], Klebanoff, MA[3], Zoh R[4], Bass D[5], Hougaard DM[6]. and Skogstrand, K[6]. 2016. Haggerty CLPregnancy Hypertens. First and second trimester immune biomerkers in preeclamptic and normotensive women 6(4):388-393. doi: 10.1016/j.preghy.2016.09.002. Epub 2016 Sep 17.

Tessa E.R. Gillon, Anouk Pels, Peter von Dadelszen, Karen mac Donell and Laura A. Mager, 2014. Hypertensive Disorders of Pregnancy. A Systematic Review of *International Clinical Practice Guidelines*. Plos One 2014; 9(12)e113715 doi 10.1371/journal.pone0013715 PMCID: PMC4249974.

Xie, L., Mouillet, J.-F., Cu T., Parks, W. T., Sadovsky, E., Knofler, M. and Sodovsky, Y. 2014. "C19MC MicroRNAs Regulate the Migration of Human Trophoblasts ". Reproduction-evelopment. 155 (12):4975–4985. doi:10.1210/en.2014-1501.

Alicia Martinez-Varea,Begona Pellicer, Alfredo Perales-Marin and Andonio Pellicer, 2014. Relationship between Maternal Immunological Response during Pregnancy and Onset of Preeclampsia. *Journal of Imunnology Research,* Article ID 210241.

****