Serum Galectin-3 in Women With Gestational Hypertension and Preeclampsia and Its Association With Preterm Birth

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Abstract- Hypertensive disorders of pregnancy are one of the leading causes of fetal and maternal mortality worldwide. Aside from the immediate risk they pose for the pregnant woman, there is significant evidence that women after such a pregnancy have a long-term risk for the development of cardiovascular diseases. On the other hand, Galectin-3 is a biomarker that has proven its role in cardiac remodeling, fibrosis, and heart failure. To determine the levels of Galectin-3 in women with gestational hypertension, preeclampsia, and in healthy pregnant women and test for association with premature birth. A prospective single-center clinical, epidemiological study was performed, and data were analyzed for 123 pregnant women-36 with gestational hypertension, 37 with preeclampsia, and 50 controls. ELISA method was used to determine the serum levels of Galectin-3. Mean Galectin-3 level was 6,53 ng/ml in the controls, 7.30 ng/ml in the gestational hypertension group, and 7,59 ng/ml in the preeclampsia group. There was a significant difference in the levels between the controls and each of the pathological groups (P<0,05), while the two pathological groups were not statistically different from each other. Additionally, higher Galectin-3 levels were associated with an OR~2.5 for even preterm birth after adjustment for the presence of the two hypertensive disorders of pregnancy. Gestational hypertension and preeclampsia were associated with significantly higher levels of Galectin-3, which could be indicative of cardiovascular dysfunction in those women, and were also related to premature birth.

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Introduction

According to reports from the World Health Organization and The International Federation of Gynecology and Obstetrics, hypertension is the most common complication of human pregnancy in the modern world, affecting approximately 5-10% of pregnancies worldwide (1,2). Much has changed about the concept of hypertensive disorders of pregnancy over the years. Initially considered to be an entirely obstetric problem, resolved with the end of the pregnancy or soon after, in the 60ties and 70ties of the previous century, scientific reports about the long-term consequences of preeclampsia started to emerge (3,4).

Besides the multisystem involvement that could be caused by preeclampsia, evidence from a vast number of cohort prospective and retrospective studies was accumulated that women after a hypertensive pregnancy were at a higher risk of developing arterial hypertension, coronary atherosclerosis, cerebrovascular disease,

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peripheral artery disease, venous thromboembolism and type 2 diabetes mellitus (5-11). The explanation was sought in the presence of shared risk factors and pathogenetic mechanisms with cardiovascular diseases, despite the fact that the pathogenesis of preeclampsia itself remains ambiguous till now. Endothelial dysfunction, thrombophilia, oxidative stress, inflammatory response, and genetic predispositions are considered to be the common denominators (8,12). Gestational hypertension seems to be the less studied of the two pathologies, probably due to its generally more benign course compared to the protein-losing preeclampsia; nonetheless research proving long-term unfavorable cardiovascular profile after gestational hypertension exists (13,14).

A particularly intriguing concept of preeclampsia as a failed "stress test" of the female organism was suggested by Kaaja *et al.*, Based on a vast literature analysis encompassing studies from 1990 to 2005 the authors stated the hypothesis that a hypertensive pregnancy potentially unmasks a predisposition of certain women to develop cardiovascular diseases later in life (15). This theory was further supported by more authors in the following years (16,17). Better understanding of why hypertensive disorders of the pregnancy are related to future heart diseases is needed. A variety of biomarkers are nowadays widely used in the field of cardiology to detect cardiac and endothelial damage in different pathologies in order to stratify risk and to guide treatment (18). They potentially represent an objective way of measuring changes happening during a hypertensive pregnancy and could enable the determination of cardiovascular involvement in those women (19). Especially useful biomarkers would be those that have already proven their role in the assessment of patients with cardiovascular diseases. Elevated levels of those markers could indicate a higher cardiovascular risk in a specific subgroup of women and this knowledge could contribute to the follow-up after such a pregnancy. Galectin-3 is one such biomarker as there is growing evidence of its role in cardiovascular diseases (20).

Objectives

The aim of this study was to determine and compare the levels of Galectin-3 in patients with gestational hypertension, preeclampsia and in healthy pregnant women for a better understating of the presence of similar mechanisms between those conditions and cardiovascular disorders. Galectin-3 levels as an indicator for the presence of the pathologies and for premature birth is also tested. Correlations between the levels of Galectin-3 and maternal characteristics are examined.

Materials and Methods

Design and study population

A prospective single-center clinical-epidemiological study was performed between August 2018 and January 2020, and 123 pregnant women were enrolled: 37 of those women covered the established criteria for preeclampsia, 36 covered the criteria for gestational hypertension, and 50 were healthy pregnant controls. The women were recruited from the Clinic of Obstetrics and Gynecology at the University multi-profile hospital, and some of the healthy controls were referred to us by local Obstetrics and Gynecology (ObGyn) practices. The study was approved by the Ethics Committee of Medical University – and all of the participants signed a written informed consent before inclusion in the study.

One hundred sixteen of the women had singleton pregnancies, and nine women had bigeminal pregnancies-4 of them had preeclampsia, 2 had gestational hypertension, and three were in the control group. Gestational age was determined using the first day of the last menstrual cycle, and in the few women who could not recollect the date, ultrasound measurements of the fetus as interpreted by an ObGyn specialist were used. The weight and height of the women were measured with standardized equipment in the Clinic of Obstetrics and Gynecology. Weight before the pregnancy was self-reported. Weight before the pregnancy was self-reported. Body mass index (BMI) and body surface area (BSI; DuBois and DuBois formula) were calculated for each woman.

Diagnosis and forms of preeclampsia

Preeclampsia was defined as newly-appeared arterial hypertension after the 20th gestational week accompanied by significant proteinuria of≥ 300 mg for 24 hours. Gestation hypertension was defined as newly-appeared arterial hypertension after the 20th gestational week with proteinuria of less than 300 mg/l for 24 hours. Hypertension was defined as office measured blood pressure of ≥140 mmHg for systolic and/or diastolic ≥90 mmHg at least twice over the course of a minimum of 4 hours apart after the patient has been sitting for at least 15 minutes before the measurement (21).

Forms of preeclampsia and gestational hypertension were designated as severe if systolic blood pressure was ≥160 mmHg and/or diastolic blood pressure was ≥110

mm Hg. Additionally, two women with levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) twice over the upper reference limit of the laboratory were also classified as severe forms. No included women had thrombocytopenia, and due to ethical reasons, no women with clinical signs of severity (pulmonary congestion, encephalopathy, epigastric pain, etc.) or HELLP syndrome were offered to participate as their condition was considered a medical emergency. Preeclampsia and gestational hypertension were defined as early forms when the elevated blood pressure was first registered before the 34th gestational week (21).

Exclusion criteria

Women with a history of chronic arterial hypertension, diabetes mellitus, any known or discovered in the course of the study heart disease, significant systemic diseases, malignancies, alcoholism, drug addictions, renal, respiratory or liver failure as well as females under 18 years of age were excluded from the study. Women whose current condition required any urgent obstetrical or other interventions were also not asked to participate in the study for ethical reasons. For the control group, women with a registered intrauterine retardation of the fetus were also not asked to participate.

Laboratory methods

Venous blood was taken in certified monovettes with a cloth activator. The blood was then allowed to cloth at 15-20^{o} C for 30 minutes and centrifuged at 3000 RPM for 10 minutes to obtain serum. The serum was then stored at -20^{o} C in accordance with the recommendations of the test kits manufacturer. Immediately before the tests were run, the serum was thawed, temperatured and carefully mixed and analysis was made according to the instructions. Galectin-3 levels were determined with double antibody ELISA Sandwich Technique (MyBioSource, Inc. San Diego, California, USA). The minimum detectable dose (sensitivity) for Galectin-3 was 0.05 ng/ml. The results were expressed as optical density (OD) at 450 nm.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25.0 (IBM SPSS Statistics for Windows, SPSS Inc., Chicago, IL, USA) and MedCalc Version 14.8.1 (MedCalc Software, Mariakerke, Belgium).

Continuous variables were tested for normality with Kolmogorov-Smirnov and Shapiro-Wilk tests. The Student's t-test, analysis of variance (ANOVA) test and Bonferroni post hoc test were used to compare the continuous variables that had normal distribution more than two independent groups with homogeneity of variances. The continuous variables with non-normal distribution were compared with the Kruskal-Wallis test and the Mann-Whitney U test. The relationship between categorical variables in cross tables was analyzed using the χ^2 test and Fisher's exact test. Correlations analysis was performed using either Pearson's correlation coefficient or Spearman's rho according to the normality of the continuous variables.

Receiver Operating Characteristics (ROC) curve analysis was carried out to determine the discriminative ability of Galectin-3 for gestational hypertension, preeclampsia, and both of them together. The evaluation of the cut-off values was performed by validation criteria for screening tests (Sensitivity, Specificity, Accuracy, *etc.*). The optimal cut-off value was obtained from the Youden index [maximum (sensitivity+specificity−1). Logistic regression was performed to explain the relationship between variables. Findings with $P<0.05$ were considered statistically significant.

Results

Various demographic, obstetric, and clinical characteristics of the women are given in Table 1. The mean age for all the women in the study was 29,93±5,71 and it ranged between 18-43 years. Mean gestational age was 33,72±4,47 weeks and it ranged between 22,00-39,29 weeks. There was no statistical difference among women with gestational hypertension, preeclampsia, and healthy controls in relation to the known confounding factors maternal age and gestational age. There was also no statistical difference among the two pathological groups for the percentage of early and severe forms either.

Among the combined group of the two hypertensive disorders, there were significantly more primigravid women than in the group of the healthy controls (49,3% vs. 24%, P=0,008). No statistical difference between normotensive and hypertensive women was observed for second (44% vs. 26%, P=0,059), third (26% vs. 17.8%, P=0,385) or more than three (6% vs. 6.8%, P=0,845) pregnancies (not given in table). This supports the notion of the first pregnancy as a well-known predisposing factor for hypertensive disorders (22).

Women in the three groups did not statistically differ

in BSA, weight gain from the beginning of the pregnancy till the day of the exam, and heart rate during the exam. Women with preeclampsia and women with gestational hypertension were with a statistically higher systolic and diastolic blood pressure and body mass index at the time of the exam. Body mass index before the pregnancy was also significantly higher in both of the hypertensive groups compared to controls (Table 1). This is consistent with previous findings that higher values of pre-pregnancy BMI and BMI during pregnancy are risk factors for the development of hypertensive disorders (23,24). There was no significant difference between the women in the three groups in relation to the percentage of current smokers, former smokers, and non-smokers, as well as in women smoking during pregnancy (Table 1).

Table 1. Comparison of the three groups of women according to maternal age, gestational age, systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), body mass index (BMI) before pregnancy, current BMI, weight gain, smoker status, smoking during pregnancy, percentage of early and percentage of severe forms of the disorders

Group/	Gestational hypertension			Preeclampsia			Controls		
Characteristics	**n**	$\overline{X}$	**SD**	**n**	$\overline{X}$	**SD**	**n**	$\overline{X}$	**SD**
Maternal age (years)	36	28,83[a]	5,78	37	29,81[a]	5,14	50	30,82[a]	6,02
Gestational age (weeks)	36	33,71[a]	4,08	37	33,24[a]	3,74	50	34,08[a]	5,23
SBP [mmHg]	33	121,91[b]	12,28	34	126,32[b]	12,20	46	107,07[a]	10,36
DBP [mmHg]	33	78,12[b]	10,91	34	83,18[b]	9,80	46	68,70[a]	7,92
HR [bpm]	35	85,66[a]	12,28	35	81,74[a]	14,11	50	86,00[a]	11,06
BMI–before pregnancy [kg/m^2]	35	28,58[b]	6,14	35	27,26[b]	5,68	49	22,58[a]	5,11
BMI–current [kg/m^2]	36	33,66[b]	5,75	36	31,77[b]	5,32	50	27,81[a]	5,49
Weight gain [kg]	35	13,69[a]	6,54	36	12,94[a]	7,51	49	14,05[a]	6,18
	n	**%**	**Sp**	**n**	**%**	**Sp**	**n**	**%**	**Sp**
Smoker **Non**	15	41,7[a]	8,2	15	40,5[a]	8,1	13	26,0[a]	6,2
Former	2	5,6[a]	3,8	3	8,1[a]	4,5	10	20,0[a]	5,7
Current	16	44,4[a]	8,3	17	45,9[a]	8,2	27	54,0[a]	7,0
Smoking during pregnancy	6	37,5[a]	12,1	8	47,1[a]	12,1	14	51,9[a]	9,6
	n	**%**	**Sp**	**n**	**%**	**Sp**	***P***		
Early forms	26	72,2	7,5	31	83,8	6,1	0,269		
Severe forms	13	36,1	8,0	13	35,1	7,8	1,000		

* -Same letters in the rows signify the lack of a statistical difference, while different letters signify the presence of a significant difference (P<0,05)

Mean Galectin-3 level was 6,53±1,87 ng/ml in the control group, 7,31±1,92 ng/ml in the gestational hypertension group, and 7,59±1,63 ng/ml in the preeclampsia group. The levels were significantly lower in the control group compared to both the gestational hypertension group (P=0,022) and to the preeclampsia group (P=0,004). Gestational hypertension and the preeclampsia group were not statistically different from each other despite a tendency for higher levels in the preeclampsia group (P=0,639). The mean Galectin-3 levels did not statistically differ between women when separated according to gravidity (1, 2, 3, or more than three pregnancies including current), smoking status (smoker, non-smoker, and former smoker), or smoking during pregnancy (Table 2).

Correlation analysis was performed, and no correlation was found between Galectin-3 levels and maternal age, BMI before pregnancy, current BMI, weight gain, the maximum registered value of systolic and diastolic blood pressure when analyzing all of the women together or separately for the three groups. A very weak negative correlation (r=-0,184, P<0,05) was found between gestational age and Galectin-3 levels only when analyzing all of the women together, but not separated into groups (Table 3).

Table 2. Comparative analysis between Galectin-3 levels and gravidity, smoking status of the women, and smoking during pregnancy

		1			2			3+	
Gravidity	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	**SD**
	48	7,20[a]	1,91	41	7,03[a]	2,08	34	6,96[a]	1,54
		Never			Former			Current	
Smoking status	n	$\overline{X}$	SD	n	$\overline{X}$	SD	n	$\overline{X}$	**SD**
	43	6,97[a]	2,06	15	7,13[a]	1,36	60	7,09[a]	1,61
		No			Yes				
Smoking during pregnancy	n	$\overline{X}$	SD	n	$\overline{X}$	SD		*P*	
	32	7,18	1,85	28	6,99	1,31		0,423	

* -Same letters in the rows signify the lack of a statistical difference, while different letters signify the presence of a significant difference (*P*<0,05)

Table 3. Correlation coefficients between Galectin-3 levels and certain characteristics of the women

Characteristics	All women	Controls	Gestational hypertension	Preeclampsia
Maternal age	0,053	0,101	-0,056	0,233
Gestational age	-0,184*	-0,175	-0,050	-0,034
BMI before pregnancy	0,085	-0,093	0,278	-0,190
Current BMI	0,051	-0,189	0,213	-0,164
BSA	-0,015	-0,116	0,028	-0,249
Current weight gain	-0,094	-0,107	-0,245	0,027
Maximum SBP	-0,063	.	-0,020	-0,108
Maximum DBP	0,070	.	0,141	-0,015

* - *P*<0,05, ** - *P*<0,01, *** - *P*<0,001,

The ROC curve of Galectin-3 for differentiating between gestational hypertension and normotensive women gave an area under the curve of 0,646 (*P*=0,022), (Figure 1A) with a sensitivity of 64% and specificity of 68%; for differentiating between preeclampsia and normotensive women AUC=0,681, (*P*=0,004), (Figure 1B) with a sensitivity of 70% and specificity of 68%; and for differentiating between the combined group of hypertensive disorders and normotensive women AUC=0,664 (*P*=0,002), (Figure 1C) with a sensitivity of 70% and specificity of 64%. The most appropriate cut-off values of Galectin-3 in each case were determined to be as close to 100% sensitivity and 100% specificity as possible and were ≥7,25 ng/ml for gestational hypertension vs. controls; ≥7,25 ng/ml for preeclampsia vs. controls, and ≥7,15 ng/ml for the combined hypertensive group vs. controls.

Binary logistic regression was used in order to quantify the relationship between the established cut-off values of Galectin-3 and the presence of gestational hypertension, preeclampsia, and either of them. Women with values of Galectin-3 ≥7,15 ng/ml were approximately four times (OR=4,121, *P*<0,001) more likely to have either of the two pathologies compared to women with Galectin-3 <7,15 ng/ml. Women with Galectin-3 levels of ≥7,25 ng/ml were approximately 3.8 times more likely to have have gestational hypertension (OR=3,760, *P*=0,004) and also approximately five times (OR=5,023, *P*=0,001) more likely to have preeclampsia than women whose levels were below 7,25 ng/ml (Table 4).

Table 4. Odds ratio and 95% CI of Galectin-3 levels as a factor related to gestational hypertension and preeclampsia

	Cut-off values (ng/ml)	OR	95% CI		*P*
			Lower	Upper	
Gestational hypertension	≥7,25/<7,25	3,760	1,524	9,276	0,004
Preeclampsia	≥7,25/<7,25	5,023	1,998	12,628	0,001
Gestational hypertension+Preeclampsia	≥7,15/<7,15	4,121	1,920	8,846	<0,001

In order to assess whether the levels of Galectin-3 had any relation to the occurrence of premature birth, birth records of all but two women (both of them in the control group) were obtained. ROC curve analysis (Figure 1D) was performed in order to establish a cut-off value of Galectin-3 for distinguishing between the women who had preterm birth (defined as birth before completed 37 gestational weeks) and term birth (after completed 37 gestational weeks) (21). The area under the curve was 0,637 (*P*=0,016) for a cut-off of ≥7,25 ng/ml with a sensitivity of 74%, specificity of 55%, a positive predictive value of 43%, and a negative predictive value of 82%. Binary logistic regression gave statistically significant crude OR~3.5 for the occurrence of premature birth if Galectin-3 levels were ≥7,25 ng/ml compared to levels <7,25 ng/ml. After adjustment for the presence of either preeclampsia or gestational hypertension, OR fell to 2,45 but remained significant. The presence of either of the two hypertensive disorders gave OR of~4,3 for the occurrence of premature birth in multivariable analysis (Table 5).

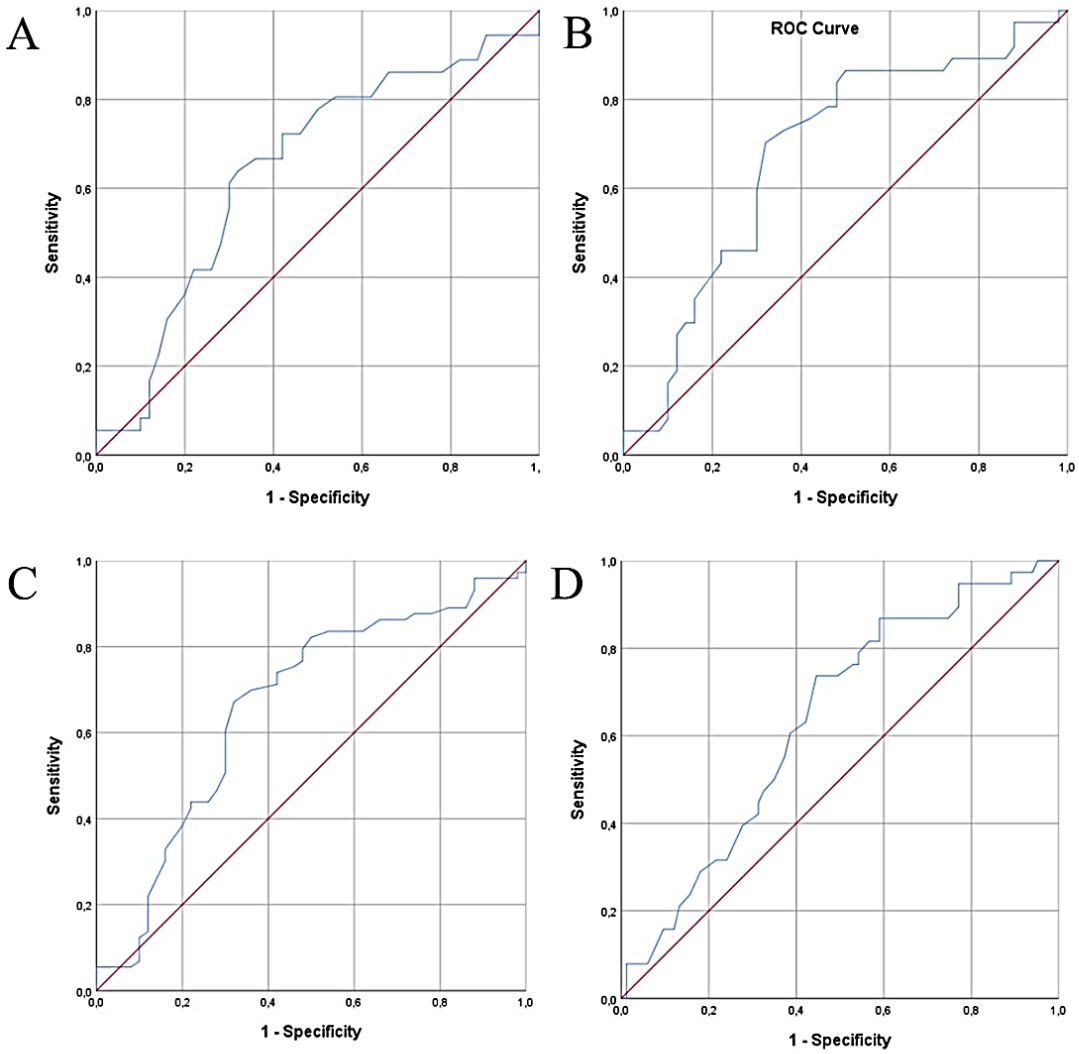

Figure 1. A) ROC curve for Galectin-3 (AUC=0,646, *P*=0,022) for determining the cut-off value for differentiating between controls and gestational hypertension group, B) ROC curve for Galectin-3 (AUC=0,681, *P*=0,004) for determining the cut-off value for differentiating between controls and the preeclampsia group, C) ROC curve for Galectin-3 (AUC=0,664, *P*=0,002) for determining the cut-off value for differentiating between controls and both of the combined hypertensive group, D) ROC curve for Galectin-3 for establishing a cut-off value for distinguishing term from preterm birth. (AUC 0,637 *P*=0,016)

Table 5. Crude and adjusted binary logistic model for Galectin-3 as a predictor for birth before completed 37 gestational weeks

Variables included in the model		Term versus preterm birth					
		Crude			Multivariate		
		OR	95% CI		OR	95% CI	
			Lower	Upper		Lower	Upper
Galectin-3 ≥ 7,25 ng/ml		3,48	1,50	8,08	2,45	1,003	5,965
Galectin-3 < 7,25 ng/ml		1,00	--	--	1,00	--	--
Groups	Controls	--	--	--	1,00	--	--
	Pathological	--	--	--	4,26	1,560	11,640

Discussion

Galectin-3 is a protein with an established role in the inflammatory process, immunity, and cancerogenesis. It is secreted by the activated macrophages and is known to activate the proliferation of fibroblasts and increase the production of collagen. Animal studies have shown its role in the process of fibrosis in the heart as well as the liver and kidneys (25). Experimental animal models have linked its activity with increased ventricular remodeling (26).

In humans, its upregulation has been proven in patients with left ventricular hypertrophy, and it also has a positive correlation with the number of hospitalizations for heart failure (27,28). Higher levels have been detected in patients with pulmonary hypertension, where they also correlate with the prognosis regardless of the etiology (29). In another study by Nar *et al*., it has been established that there is a correlation between Galectin-3 levels and septal and posterior wall thickness of the left ventricle as well as left ventricular mass. In the same study, its levels were elevated even in the setting of new-diagnosed hypertension (30).

In an experimental study by Li *et al*., administration of Galectin-3 in mice caused insulin resistance and glucose intolerance. The authors suggest that a Galectin-3-related connection between inflammation and decreased sensitivity to insulin exists (31). A link has been established between Galectin-3 levels and obesity, insulin resistance, type 2 diabetes in humans as well. Its role as a novel marker and therapeutic target in metabolic diseases has also been suggested (32).

Very scarce information is available on Galactin-3 in hypertensive pregnacies. We identified a relevant 2020 study conducted by Taha *et al*., in which 60 Iraqi women with preeclampsia and 30 healthy pregnant controls were examined and the levels of Galectin-3 similarly to our study, were significantly higher in the preeclamptic women. Additionally, in their study the levels of Galectin-3 also correlated with a more unfavorable lipid profile in the women. Moderate positive correlation between serum Galectin-3 levels and duration of pregnancy as well as maternal age was established for the women with preeclampsia. That last finding is not confirmed by our study as no correlation with maternal age was established, and only a very weak negative correlation exists between its levels and gestational age at inclusion (33).

An immunohistochemical study published in 2007 by Jeschke *et al*., showed a significant up-regulation of the expression of both galectin-1 and galectin-3 in the extravillous trophoblast of the placentas of 8 women with preeclampsia and 5 with HELLP syndrome compared to 8 normal placentas (34). A study by Demmert et al. published in 2012 analyzed 21 healthy terms and 125 preterm neonates and found a significantly higher expression of Galectin-3 in the umbilical cord blood of small-for-gestational-age infants compared to appropriate-for-gestational-age ones (35).

We are currently not aware of the existence of another study providing ROC curves for Galectin-3 for distinguishing between preeclampsia and gestational hypertension and healthy pregnant women and establishing a cut-off value, its sensitivity, specificity, percentage of correct answers, and odds ratio. We could not identify another study in which levels of Galectin-3 were examined, particularly in women with gestational hypertension.

In our study, higher Galectin-3 levels were also associated with a significantly higher risk of preterm birth even after adjustment for the presence of preeclampsia and gestational hypertension. Miyaauchi *et al*., created a model of mice infected by *Porphyromonas gingivalis*, which mimicked chronic dental infection in pregnant women. A connection between higher levels of Galectin-3 as an inflammation mediator and preterm birth was established in this model (36).

Galectin-3 could prove to be a marker independently reflecting the presence of the two hypertensive disorders as in our study population. Its levels did not correlate with the majority of the characteristics of the mother

(age, BMI before pregnancy, current BMI, weight gain, maximum recorded systolic and diastolic blood pressure), and correlation with gestational age was statistically negligible. Means levels of Galectin-3 had no statistical difference when the women were separated according to smoking status, smoking during pregnancy, and gravidity.

The connection between higher Galectin-3 levels and the presence of different cardiovascular pathologies in non-pregnant populations could be taken into account when interpreting the results of our study. If we follow the theory of hypertensive pregnancy as a failed stress test (15), the significantly higher galectin-3 levels in those women could mean that Galectin-3 is a potential biomarker that reflects the eventual occurrence of arterial hypertension, ventricular remodeling, and metabolic disturbances after such a pregnancy. Further research is needed to establish if the higher levels are part of the cause or the result of those pregnancy disorders. Additional studies of Galectin-3 levels in women with a history of preeclampsia and gestational hypertension could determine whether significantly higher levels persist later in their lives and for how long.

Galectin-3 levels were significantly higher in pregnancies, complicated by both preeclampsia and gestational hypertension compared to normotensive ones. Levels higher than the provided cut-offs in our study determined 3,8 to 5-fold higher risk for the presence of preeclampsia or gestational hypertension in pregnant women. Higher levels of Galectin-3 also predicted the occurrence of preterm birth with an OR of 2.45 after adjustment for preeclampsia and gestational hypertension. Its levels were independent of the majority of patients' characteristics and had a negligible correlation with gestational age. Galectin-3, which has an established role in inflammation and a variety of cardiovascular conditions, could potentially be a missing link between hypertensive disorders of pregnancy and elevated future cardiovascular risk in women.

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