ORIGINAL ARTICLE

Maternal serum levels of neutrophil gelatinase-associated lipocalin (NGAL), matrix metalloproteinase-9 (MMP-9) and their complex MMP-9/NGAL in pregnancies with preeclampsia and those with a small for gestational age neonate: a longitudinal study

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ABSTRACT

Background The aim of this study was to determine maternal serum concentrations of neutrophil gelatinase-associated lipocalin (NGAL), matrix metalloproteinase-9 (MMP-9), and MMP-9/NGAL complex longitudinally in pregnancy, in normal pregnancies, in pregnancies that developed preeclampsia and in pregnancies that delivered a small for gestational age infant (SGA).

Methods Neutrophil gelatinase-associated lipocalin, MMP-9, and MMP-9/NGAL were determined in the first, second, and third trimesters in 33 normal pregnancies, 12 pregnancies complicated by preeclampsia, and 14 pregnancies that delivered a SGA neonate.

Results Median NGAL concentration (ng/mL) in normal pregnancies increased significantly from 12.8 in the first trimester to 25.9 in the second trimester ($p = 0,002$) and 48.0 ($p < 0.0001$) in the third trimester. In preeclamptic pregnancies, NGAL was significantly higher, compared with normal pregnancies, in the first (30.9; $p = 0.006$) and second (44.6; $p = 0.015$) trimesters. MMP-9 and MMP-9/NGAL complex concentrations in preeclamptic pregnancies did not differ significantly from normal pregnancies in either trimester. Pregnancies with an SGA infant did not have different marker concentrations in either trimester, compared with normal pregnancies.

Conclusion Maternal serum NGAL, MMP-9, and MMP-9/NGAL complex concentrations tend to increase during pregnancy in normal and preeclamptic pregnancies. NGAL was significantly elevated in the first and second trimesters, in pregnancies that later developed preeclampsia. © 2014 John Wiley & Sons, Ltd.

INTRODUCTION

Preeclampsia is a systematic disease of pregnancy characterized by hypertension and proteinuria developing after the 20th week of pregnancy. It is estimated that preeclampsia affects 3–5% of all pregnancies worldwide and is one of the most frequently encountered medical complications of pregnancy.[1] In Greece, the estimated incidence of preeclampsia (2.8%) is comparable with the worldwide incidence.[2]

The pathogenesis of preeclampsia is still not fully understood despite the intense research on the field.[3] Abnormal cytotrophoblastic invasion of spiral arterioles, reduced or insufficient placental perfusion, and the subsequent maternal endothelial dysfunction seems to be central to the pathophysiology of the syndrome.[4,5]

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein of the lipocalin superfamily. Human NGAL was originally identified as a protein isolated from the secondary granules of human neutrophils.[6,7] Human NGAL consists of a single polypeptide chain of 178 amino acid residues with a calculated molecular mass of 22 kDa and it occurs mainly as a monomer, with a small proportion as dimer and trimer. It has been demonstrated that NGAL also occurred as a complex covalently linked with the 92-kDa matrix metalloproteinase-9 (MMP-9) also known as gelatinase B.[8,9]

Studies in the early 1990s started to explore the possible roles of NGAL. NGAL mRNA is normally expressed in a variety of human tissues including the kidney, bone marrow, prostate, uterus, salivary gland, stomach, colon, lung, and the liver. Neutrophils, monocytes/macrophages, and adipocytes are the cells with abundant NGAL expression. Consequently, NGAL seems to have a crucial role in numerous physiological and pathological roles including antibacterial activity, embryogenesis, neoplastic growth, renal-cardiovascular disease, and epithelium function.[10]

There are only few studies that examine maternal serum levels of NGAL during normal or pathological pregnancy. The first study on NGAL in pregnancy was published in 2008 and reported elevated concentrations of NGAL in pregnancies that later developed preeclampsia compared with normal pregnancies in late second trimester (24–26 weeks).[11] The same group of researchers later reported elevated NGAL concentrations in preeclamptic pregnancies in all trimesters of pregnancy.[12,13] These results were also confirmed by the study of Stepan H *et al.*[14] Interestingly, in a recent study, NGAL together with placental growth factor and soluble fms-like tyrosine kinase-1 (sFlt-1) was among the most effective biomarkers in the first trimester for the prediction of late preeclampsia.[15] On the contrary, a recent study from Cemgil Arikan D *et al.*,[16] reported lower levels of maternal NGAL in preeclamptic compared with normal pregnancies. It is worth to be mentioned that there is no study on the levels of NGAL in pregnancies that had a small for gestational age infant (SGA) or on MMP-9/NGAL complex in normal or pathological pregnancies.

Matrix metalloproteinase-9 is a zinc-dependent proteinase involved in inflammation, tissue remodeling, mobilization of matrix-bound growth factors, and processing of cytokines.[17,18] In pregnancy, maternal concentration of MMP-9 is about 15 times higher than in non-pregnant women.[19] The enzyme may be involved in trophoblastic invasion and placentation as it is well-documented that there exists a decreased expression of MMP-9 and other metalloproteinases in preeclamptic placenta. This may lead to impaired invasion of trophoblast cells, causing abnormal placentation and occurrence of preeclampsia.[20–24] Nonetheless, there is a controversy on the levels of MMP-9 in preeclamptic pregnancies compared with normal ones. Many studies reported higher levels in preeclampsia, whereas in other studies, there is no statistically significant difference, or in some cases, even lower levels compared with normal pregnancies have been reported.[25–28]

The aim of this study was to determine maternal serum concentrations of NGAL, MMP-9, and MMP-9/NGAL complex longitudinally in pregnancy, in normal pregnancies, in pregnancies that developed preeclampsia, and in pregnancies that delivered an SGA infant.

MATERIALS AND METHODS

The pregnant women included in the present study were selected from a pool of pregnancies that are recruited for a wider ongoing investigation project on biochemical and ultrasound markers for the development of adverse pregnancy outcomes. Pregnancies are recruited in the study in the first trimester as they appear for the routine prenatal screening for chromosomal abnormalities, in the Second Department of Obstetrics and Gynecology of Medical School of Athens University in Aretaieio Hospital and in a private setting of obstetric care (EmbryoCare, Fetal Medicine Unit, Athens, Greece). All women gave their informed consent for their participation in the study and the hospital's ethics committee approved the protocol.

From a total of 541 women with known pregnancy outcome, in this case–control study, we included all 12 singleton pregnancies that developed preeclampsia. One preeclamptic twin pregnancy was excluded from the study. Preeclampsia was defined as hypertension (systolic blood pressure =140 mmHg and/or diastolic blood pressure > = 90 mmHg) and proteinuria (protein concentration > = 0.3 g per 24-h urine collection) developed after the 20th week of pregnancy. Blood pressures were averages of two or more measurements. Out of the 12 pregnancies with preeclampsia, five delivered before or at the 35th week of gestation and the remaining seven after the 35th week of gestation. Clinical characteristics of each one of the women who developed preeclampsia and their infants are shown in Table 1.

From the pool of 541 pregnancies, we randomly selected 33 women with singleton normal pregnancies that did not have any major complication during their pregnancy (diabetes, hypertension, and preeclampsia) and delivered healthy full-term neonates. From the same pool of pregnancies, we also randomly selected 14 women that delivered an SGA infant. SGA infants were defined by infant birth weight ≤10th percentile of the corresponding curves after adjustment for gestational age and gender.[29] Pregnancies with SGA infants did not develop any pathology (hypertension, preeclampsia, or diabetes) and had normal amniotic fluid volume and normal umbilical artery Doppler in the third trimester. Demographic and clinical characteristics of the three studied groups of pregnancies as well as their newborns are shown in Table 2.

Blood samples and ultrasonographic and Doppler data were collected prospectively in the first trimester (11–14th week) during the nuchal translucency scan, in the second trimester (20–26th week) during the anomaly scan, and in the third trimester (28–35th week) during the growth scan. Serum samples were aliquoted and stored at −35 °C until analysis.

In the three groups of women, preeclamptic, normal, and SGA, the concentrations of NGAL, MMP-9, and MMP-9/NGAL complex were retrospectively determined in the first, second, and third trimesters. Determinations were performed with the R&D (R&D Systems Inc, MN, USA) kits: Human Lipocalin-2/NGAL Quantikine; Human MMP-9 Quantikine ELISA; Human MMP-9/NGAL Complex Quantikine ELISA. According to the kits' inserts, the detection limit was 0.012 ng/mL for NGAL, 0.156 ng/mL for MMP-9, and 0.058 ng/mL for the MMP-9/NGAL complex, whereas the precision, as it is estimated by the total % coefficient of variation (CV) was 7.9% for NGAL, 7.9% for MMP-9, and 7.6% for the MMP-9/NGAL complex.

The statistical software: SPSS statistics version 17 (IBM Corporation, Somers, NY 10589, USA) was used for data

Table 1 Demographic and clinical characteristics of the 12 pregnancies complicated with preeclampsia and their newborns

								Uterine artery Doppler PI			
a/a	Age (years)	BMI at First trimester	Mode of delivery	GA at delivery (week + days)	Sex	BW (g)	BW centile (%)	First trimester	Second trimester	Third trimester	Average blood pressure at third trimester
1	28.2	35.7	CS	32 + 3	Boy	1665	14.7	1.72	1.14		150/100
2	27.6	22.8	CS	37 + 2	Boy	2010	0.3	1.70	1.75		140/90
3	38.6	32.0	CS	38 + 4	Girl	3800	90.6	1.47	0.88	0.62	140/84
4	32.0	23.1	CS	38 + 5	Boy	3240	40.8	1.44	0.84	0.86	161/72
5	34.2	33.3	CS	31 + 1	Boy	1040	1.7	1.21	0.95	1.12	140/82
6	36.6	23.1	VD	38 + 2	Girl	2260	0.8	2.09	1.98	1.11	141/84
7	38.1	23.9	CS	37 + 4	Girl	2760	23.8	1.11	0.99	0.62	168/87
8	34.7	28.5	VD	38 + 2	Girl	3270	53.8	1.6	0.96	0.86	133/97
9	34.2	30.0	CS	35 + 0	Girl	1315	0.3	1.81	1.14	1.33	142/89
10	35.8	21.1	CS	30 + 2	Boy	930	2.3	2.55	2.0	2.04	142/78
11	38.8	32.0	—	34 + 4	Girl	1915	11.8	1.8	1.13	0.82	129/94
12	33.4	31.9	CS	36 + 0	Girl	2150	12.4		1.10		145/90

PI, pulsatility index; BMI, body mass index; GA, gestational age; BW, birthweight; CS, cesarean section; VD, vaginal delivery.

Table 2 Demographic and clinical characteristics of the studied pregnancies and their newborns

Characteristic	Normal pregnancies	Pregnancies complicated with preeclampsia	Pregnancies with SGA infants
N	33	12	14
Maternal age at first trimester (y)	32.5 ± 3.8	34.3 ± 3.7	30.7 ± 4.7*
Parity (% nulliparus)	46%	67%	50%
Smokers (%)	15%	25%	21%
Maternal BMI at first trimester (Kg/m^2)	24.1 ± 4.0	28.1 ± 5.0**	24.6 ± 4.7
Doppler PI of uterine artery			
First trimester	1.61 ± 0.39	1.68 ± 0.40	1.75 ± 0.53
Second trimester	0.97 ± 0.32	1.24 ± 0.42**	1.0 ± 0.24
Third trimester	0.76 ± 0.14	1.04 ± 0.44***	0.82 ± 0.14
Mean blood pressure at third trimester			
Systolic (mmHg)	111 ± 8	144 ± 11#	105 ± 9
Diastolic (mmHg)	67 ± 7	87 ± 8#	65 ± 7
Gestational age at delivery (weeks)	39.2 ± 1.0	35.7 ± 3.0#	39.1 ± 0.8
Birthweight (g)	3344 ± 288	2196 ± 917##	2663 ± 222##
Birthweight percentile (median, range)	43 (11–97)	21 (1–91)###	7 (1–10)##

SGA, small for gestational age infant; BMI, body mass index; PI: pulsatility index.
*$p = 0.04$ compared with preeclamptic pregnancies.
**$p = 0.03$ compared with normal pregnancies.
***$p = 0.05$ compared with normal pregnancies.
#$p < 0.001$ compared with normal and SGA pregnancies.
##$p < 0.001$ compared with normal pregnancies.
###$p = 0.003$ compared with normal pregnancies.

analysis. NGAL, MMP-9, and MMP-9/NGAL complex concentrations were normally distributed in normal pregnancies but not in preeclamptic and pregnancies with SGA infants in some trimesters, as checked with Shapiro–Wilk test. Comparisons of NGAL, MMP-9, and MMP-9/NGAL complex concentrations between trimesters and patients groups were performed by non-parametric tests (Kruskal–Wallis or Mann–Whitney test). Comparisons of quantitative data were performed by t-test and of qualitative data by chi-square (χ^2) test. Correlations were evaluated using Pearson's correlation coefficient. Comparisons of percentiles were carried out by Mann–Whitney U-test. Data are presented as median and interquartile range in parenthesis. A probability level of less or equal to 0.05 was considered significant.

RESULTS

There was no statistically significant difference in maternal age between women with normal and preeclamptic pregnancies. However, maternal age of women that delivered an SGA infant was significantly lower ($p=0.04$) than that of women with preeclamptic pregnancies (Table 2). Maternal body mass index (BMI), calculated in the first trimester, was higher in women who developed preeclampsia ($p=0.03$) compared with women with normal pregnancies. The three groups of women did not differ significantly in relation to parity and smoking. Mean pulsatility index (PI) estimated by uterine artery Doppler ultrasound was found significantly elevated in women with preeclampsia compared with normal pregnancies both in the second ($p=0.03$) and third trimesters ($p=0.05$) but not in the first trimester. PI did not differ between normal pregnancies and pregnancies with SGA fetuses in either trimester. Women with preeclampsia had significantly higher systolic and diastolic blood pressure in the third trimester ($p<0.001$), as it is predictable, compared with women of the two other groups. The mean gestational age at delivery did not differ between normal pregnancies and pregnancies with SGA infants but it was more than 3 weeks earlier in pregnancies with preeclampsia ($p<0.001$). Average birth weight of neonates from women who developed preeclampsia as well as from women with SGA infants were significantly lower ($p<0.001$) than that of neonates from normal pregnancies. Median birth weight percentile of SGA neonates was, by definition, significantly lower ($p<0.001$) than the median birth weight percentile of neonates from normal pregnancies. The median birth weight percentile of neonates born by preeclamptic women was also lower ($p=0.003$) from that of normal pregnancies.

Neutrophil gelatinase-associated lipocalin, MMP-9, and MMP-9/NGAL complex concentrations in normal pregnancies, in pregnancies that developed preeclampsia, and in pregnancies with SGA infants are presented in Table 3 and depicted in Figures 1–3. There was no significant difference in gestational age at the time of sampling between the three studied groups in either trimester.

Median NGAL concentration (ng/mL) in normal pregnancies increased from 12.8 (8.0–21.1) in the first trimester to 25.9 (14.7–34.6) in the second trimester and 48.0 ng/mL (22.7–78.6) in the third trimester. The difference between the first and second trimesters and between the first and third trimesters was statistically significant ($p=0.002$ and $p<0.001$, respectively). In preeclamptic pregnancies, median NGAL concentration also increased during pregnancy from 30.9 (21.0–52.3) in the first trimester to 44.6 (35.7–71.8) in the second trimester and 87.6 (43.5–115.6) in the third trimester, but the difference was significant only between the first and third trimesters ($p=0.05$). Although there was an increase in NGAL concentration in pregnancies that had an SGA infant from the first (18.9; 11.2–30.1) to the third trimester (28.4; 23.1–56.8), this difference was not statistically significant.

In the first trimester, NGAL concentration was significantly higher in pregnancies with preeclampsia than that in normal

Table 3 Median (interquartile range) concentrations of neutrophil gelatinase-associated lipocalin (NGAL, ng/mL), matrix metalloproteinase-9 (MMP-9; ng/mL), and matrix metalloproteinase-9/neutrophil gelatinase-associated lipocalin complex (MMP-9/NGAL; ng/mL) in the three studied groups, in each trimester of pregnancy

	Normal pregnancies	Pregnancies complicated with preeclampsia	Pregnancies with SGA infants
N	33	12	14
First trimester			
GA at sampling (weeks)	12.6 ± 0.5	12.4 ± 0.3	12.6 ± 0.5
NGAL	12.8 (8.0–21.1)	30.9 (21.0–52.3)[#]	18.9 (11.2–30.1)
MMP-9	640 (400–846)	622 (517–910)	601 (462–1025)
MMP-9/NGAL	54.6 (30.3–136.3)	103.8 (76.6–201.7)	35.4 (25.3–122.1)
Second trimester			
GA at sampling (weeks)	21.6 ± 0.7	21.6 ± 1.1	21.8 ± 1.3
NGAL	25.9 (14.7–34.6)*	44.6 (35.7–71.8)[##]	21.6 (12.6–58.0)
MMP-9	670 (417–923)	732 (594–952)	609 (362–1017)
MMP-9/NGAL	82.0 (60.0–137.6)	152.8 (101.4–192.2)	60.4 (40.4–237.6)
Third trimester			
GA at sampling (weeks)	31.7 ± 1.2	31.8 ± 2.1	31.4 ± 1.2
NGAL	48.0 (22.7–78.6)**	87.6 (43.5–115.6)***	28.4 (23.1–56.8)
MMP-9	1164 (667–1618)**	1382 (688–1777)***	822 (391–1558)
MMP-9/NGAL	237.4 (146.5–422.0)**	309.1 (249.2–494.4)**	52.2 (39.2–452.4)

GA, gestational age (mean ± SD).
*$p=0.002$ compared with first trimester.
**$p<0.01$ compared with first and second trimester.
***$p\leq0.05$ compared with first trimester.
[#]$p=0.006$ compared with normal pregnancies.
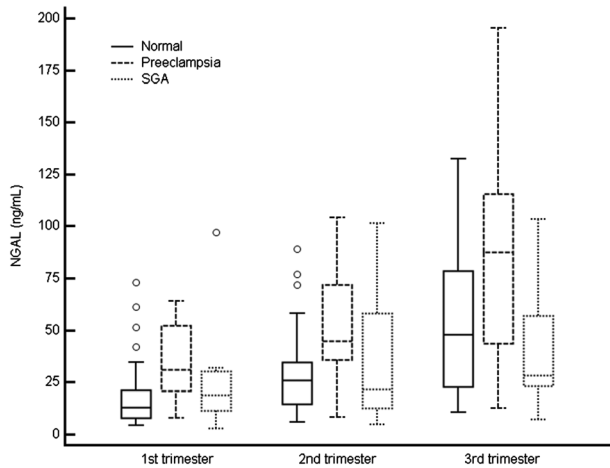[##]$p=0.015$ compared with normal pregnancies.

Figure 1 Box plots (horizontal line: median; box: 25–75% percentiles; whiskers: min–max; circles: outliers) of neutrophil gelatinase-associated lipocalin (NGAL) values (ng/mL) in the three trimesters of pregnancy. Solid line: normal pregnancies; dash line: pregnancies complicated with preeclampsia; dot line: pregnancies with small for gestational age infants
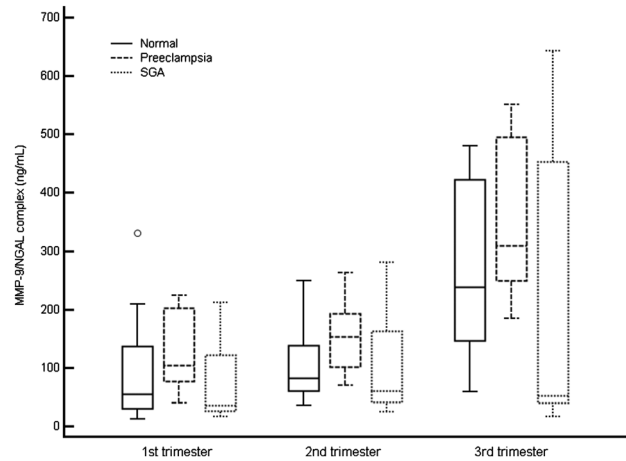

Figure 3 Box plots (horizontal line: median; box: 25–75% percentiles; whiskers: min–max; circles: outliers) of matrix metalloproteinase-9/ neutrophil gelatinase-associated lipocalin complex (MMP-9/NGAL) values (ng/mL) in the three trimesters of pregnancy. Solid line: normal pregnancies; dash line: pregnancies complicated with preeclampsia; dot line: pregnancies with small for gestational age infants
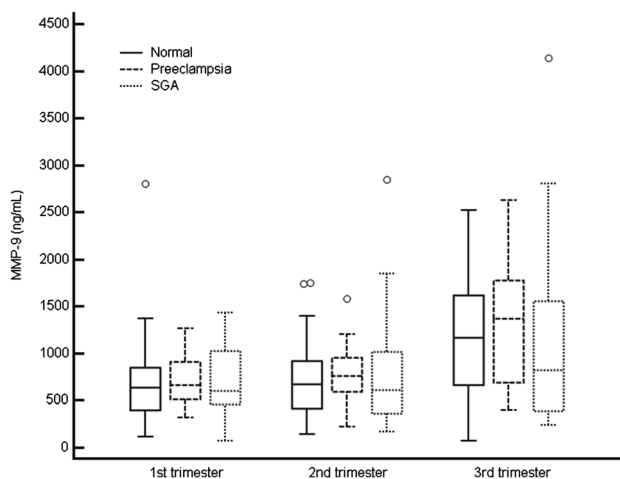

Figure 2 Box plots (horizontal line: median; box: 25–75% percentiles; whiskers: min–max; circles: outliers) of matrix metalloproteinase-9 (MMP-9) values (ng/mL) in the three trimesters of pregnancy. Solid line: normal pregnancies; dash line: pregnancies complicated with preeclampsia; dot line: pregnancies with small for gestational age infants

Median MMP-9 concentration (ng/mL) in normal pregnancies increased from 640 (400–846) in the first trimester to 670 (417–923) in the second trimester and 1164 (667–1618) in the third trimester. The difference between the first and third trimesters and the second and third trimesters was found to be statistically significant ($p = 0.001$ and $p = 0.007$, respectively). In preeclamptic pregnancies, median MMP-9 concentration also increased during pregnancy from 622 (517–910) in the first trimester to 732 (594–952) in the second trimester and 1382 (688-1777) in the third trimester. The difference between first and third trimesters was statistically significant ($p = 0.031$). Although there was an increase in MMP-9 concentration in pregnancies that had an SGA infant from the first trimester (601; 462–1025) to the third trimester (822; 391–1558), this difference was not statistically significant.

We did not find significant differences in MMP-9 concentration among the three groups in either trimester.

Finally, median MMP-9/NGAL complex concentration (ng/mL) in normal pregnancies increased from 54.6 (30.3–136.3) in the first trimester to 82.0 (60.0–137.6) in the second trimester and 237.4 (146.5–422.0) in the third trimester. The difference between the first and third and also the second and third trimesters was found to be statistically significant ($p = 0.001$ and $p = 0.003$). In preeclamptic pregnancies, median MMP-9/NGAL complex concentration also increased during pregnancy from 103.8 (76.6–201.7) in the first trimester to 152.8 (101.4–192.2) in the second trimester and 309.1 (249.2–494.4) in the third trimester. The differences between first and third trimesters and also between the second and third trimesters were found to be statistically significant ($p = 0.005$ and $p = 0.01$, respectively). In pregnancies that had an SGA infant, MMP-9/NGAL complex concentration was found to be 35.4 (25.3–122.1) in the first trimester, 60.4 (40.4–237.6) in the second trimester, and 52.2 (39.2–452.4) in the third trimester.

In all three trimesters, MMP-9/NGAL complex concentration was higher in pregnancies with preeclampsia compared with

pregnancies ($p = 0.006$). The difference between normal pregnancies and pregnancies with SGA infants was not statistically significant.

In the second trimester, NGAL was also found significantly higher in pregnancies with preeclampsia ($p = 0.015$) and lower but not statistically significant in pregnancies with SGA infants compared with normal pregnancies.

In the third trimester, NGAL was higher in pregnancies with preeclampsia compared with normal pregnancies, but the difference marginally did not reach statistical significance ($p = 0.09$). In pregnancies with SGA infants, NGAL concentration was again lower but not statistically significant.

both normal and pregnancies with SGA infants but the difference marginally did not reach statistical significance in the first and second trimesters ($p = 0.071$ and $p = 0.081$, respectively).

In normal pregnancies, we found a very strong correlation between NGAL and MMP-9/NGAL complex concentrations in the first ($r = 0.93$; $p < 0.0001$), second: ($r = 0.88$; $p < 0.0001$), and third trimesters ($p = 0.9$; $p < 0.0001$). A strong correlation was also found between MMP-9 and MMP-9/NGAL complex concentrations in the three trimesters (first: $r = 0.81$, $p < 0.0001$; second: $r = 0.89$, $p < 0.0001$; and third: $r = 0.86$, $p < 0.0001$). No correlation was found between each one of the biochemical parameters and the infant's birth weight.

DISCUSSION

In the present study, we measured NGAL, MMP-9, and MMP-9/NGAL concentrations in the first (11–14th week), second (20–26th week), and third (28–35th week) trimesters of pregnancy in normal pregnancies as well as in pregnancies complicated with preeclampsia and in pregnancies who gave birth to SGA infants. We found an increase trend of NGAL, MMP-9, and MMP-9/NGAL complex concentrations in normal pregnancies along with gestational age. The same pattern of increase was also noticed in both preeclamptic and SGA pregnancies.

In the case of NGAL in normal pregnancies, the increase from the first to the second and the third trimesters was statistically significant. In the study of D' Anna et al.,[13] the authors also found an increase trend of NGAL concentration from the first (median 13.6 ng/mL; interquartile range: 9.1–19.9) to the second (16.3 ng/mL; 11.3–23.3) and the third trimesters (median 15.8 ng/mL; 9.1–22.5) in normotensive pregnancies, but in contrast to our study, this increase was not significant. In preeclamptic pregnancies, NGAL concentration was higher in the third ($p = 0.05$) compared to the first trimester, which was also observed in the study of D' Anna et al.[13]

Regarding MMP-9, we also observed an increase trend along with gestational age in all three studied groups. Nevertheless, the difference was statistically significant only in normal and preeclamptic pregnancies between the first and third trimesters. As long as it refers to MMP-9/NGAL complex, there was also an increase trend during pregnancy, especially in the third trimester of normal and preeclamptic pregnancies, and this finding is mentioned for the first time.

The observed increase of NGAL as well as MMP-9 and MMP-9/NGAL complex concentration in normal pregnancies, as the pregnancy advances, may be a physiological contribution to moderation of MMP-9 function. It can be hypothesized that a balance among MMP-9, NGAL, and their complex is crucial and one of the possible mechanisms leading to undisturbed trophoblast invasion and thus, to normal placental perfusion. Consequently, the disturbance of this sensitive balance in the beginning of pregnancy and placentation may be one of the reasons of abnormal cytotrophoblastic invasion of the extracellular matrix and spiral arterioles in preeclamptic pregnancies.

Our results, regarding the elevated NGAL concentration during the first and second trimesters of pregnancies that later developed preeclampsia compared with normal pregnancies, are in agreement with several other studies.[11–15] As in the study of Youssef A et al.,[15] we also found elevated Doppler PI in the second trimester, together with elevated NGAL concentration in pregnancies that later developed preeclampsia. It is likely that the dysfunctional maternal endothelium, which is the result of poor placentation and impaired pseudovasculogenesis, is one of the sources of the elevated amounts of NGAL in preeclamptic pregnancies. In contrast, Cemgil Arikan D et al. noticed lower NGAL concentration in preeclamptic pregnancies compared with normal ones.[16]

In our study, we found a marginally significant increase of NGAL concentration during the third trimester, in pregnancies with preeclampsia compared with normal pregnancies. This finding is in disagreement with earlier studies that have found statistically significant elevated NGAL levels in preeclamptic pregnancies in the third trimester.[13] A likely explanation of our findings could be the small number of samples from preeclamptic women in the third trimester (7 instead of 12 and 10 in the first and second trimesters, respectively). Moreover, two of the three missing samples in the third trimester are from pregnancies that delivered very early (Table 1, cases 1 and 5) and it is possible that pregnancies with early onset preeclampsia might have more clearly elevated NGAL concentrations in the third trimester.[30]

The finding of our study concerning the lack of difference of MMP-9 between normal and preeclamptic pregnancies in any trimester is in agreement to previous studies like that from Myers JE et al.[27] It seems that MMP-9 is not a good marker for preeclampsia.[19]

In the present study, we did not found significantly different NGAL, MMP-9, or MMP-9/NGAL complex concentrations in pregnancies with SGA infants compared with normal pregnancies in any trimester. To our knowledge, this is a novel finding. These pregnancies had a normal ultrasound assessment in all three trimesters, their growth pattern was stable, and thus, the low birth weight of their infants could rather be attributed to genetic factors. Because, as we observed in the present study, the rate of increase of NGAL, MMP-9, and MMP-9/NGAL levels with the progress of gestation in normal and SGA pregnancies are similar, the SGA pregnancies of our study could be considered as normal pregnancies rather than pathological ones.

Neutrophil gelatinase-associated lipocalin, MMP-9, and MMP-9/NGAL complex concentrations in the first, second and third trimesters did not show any correlation with birth weight in normal pregnancies. This finding may imply that factors involved in prenatal growth acquisition and birth weight determination do not affect concentration of the three markers.

The strong correlation that we found in our study in normal pregnancies between NGAL and MMP-9/NGAL complex and also between MMP-9 and MMP-9/NGAL complex shows that NGAL and MMP-9 are the main determinants of the complex concentration in all trimesters.

The findings of our study may indicate that preeclampsia influences NGAL and MMP-9 concentrations by a complicated mechanism, different in each trimester. In the first and second

trimesters of a preeclamptic pregnancy, oxidative stress caused by the ischemic placenta, the result of the ongoing impaired pseudovasculogenesis, could be responsible for the elevated NGAL, MMP-9 levels through endothelial dysfunction. In the third trimester, the impaired renal function due to established endothelial damages may represent an additional mechanism, which also contributes to the elevated levels of these markers in preeclamptic pregnancies.

Our study has a number of limitations. These include (1) the small number of pregnancies with preeclampsia and especially the small number of samples from preeclamptic pregnancies in the third trimester of pregnancy and (2) missing data on pregnancies with intrauterine growth restricted (IUGR) infants without preeclampsia. These data could be useful to clarify the difference in NGAL, MMP-9, and MMP-9/NGAL complex concentrations between IUGR and SGA infants.

In conclusion, maternal serum NGAL, MMP-9, and MMP-9/NGAL complex concentration tends to increase from the first to the third trimester of normal pregnancy. We have found significantly elevated NGAL concentrations in the first and second trimesters, in pregnancies that later developed preeclampsia compared with normal pregnancies. NGAL values in the first or second trimester of pregnancy seem to be a promising marker for the prediction of preeclampsia. An important finding of our study is that pregnancies that gave birth to SGA infants did not have significantly different NGAL, MMP-9, or MMP-9/NGAL complex levels in the three trimesters of pregnancy compared with normal pregnancies.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Maternal serum NGAL and MMP-9 levels increase during pregnancy in both normal and preeclamptic pregnancies. Preeclamptic pregnancies had elevated NGAL levels in the first and second trimesters, compared with normal pregnancies.

WHAT DOES THIS STUDY ADD?

- Our study confirms that NGAL levels are elevated in preeclamptic pregnancies at least in the first and second trimesters, compared with normal pregnancies. The MMP-9/NGAL complex is measured for the first time throughout pregnancy in normal and preeclamptic pregnancies. Pregnancies that delivered SGA neonates are considered as a separate group for the evaluation of NGAL, MMP-9, and their complex.

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