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Midkine levels decrease in late preeclampsia

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Summary

Aim: The aim of this study was to determine midkine (MK) expression in women with preeclampsia (PE) compared with those in a normal pregnancy. *Materials and Methods:* This study included 45 patients with PE and 35 patients in a control group. Women with PE who had a diagnosis of preeclampsia before gestational week 34 were included in the early PE group (n = 20). Women diagnosed with PE after gestational week 34 were in the late PE group (n = 45). Serum MK levels were compared between the groups. *Results:* MK level was neither different between normotensive women and those with early PE nor was it different between the early and late PE groups. MK levels were significantly different between the normal pregnancy and late PE groups. The MK level decreased in the late PE group compared with that in the normal pregnancy group. *Conclusion:* Serum MK levels significantly decrease in late PE women compared with normally pregnant women.

Key words: Midkine; Preeclamsia; Pregnancy.

Introduction

Preeclampsia (PE) affects approximately 3% of all pregnancies [1]. Women with PE are at an increased risk for life-threatening events, including placental abruption, acute kidney injury, cerebral hemorrhage, hepatic failure or rupture, pulmonary edema, disseminated intravascular coagulation, and progression to eclampsia. PE remains a major cause of death during pregnancy worldwide, accounting for 10–15% of direct maternal deaths overall [2]. Therefore, screening tests to identify women at risk are of great potential utility. The etiology of PE is unknown, but inadequate placental development is a typical feature [3]. It is thought to be an early placental dysfunction [4], characterized by insufficient invasion of the spinal arteries by the trophoblast, placental ischemia [5], and impaired perfusion of the uteroplacental unit. In addition, systemic endothelial dysfunction, inflammation, and immunological and genetic factors have been proposed.

Midkine (MK) is a heparin-binding growth factor first discovered as a highly expressed gene during mouse embryogenesis [6]. It can be quantitated readily in blood samples, thereby making it a minimally invasive biomarker for detecting, monitoring, and managing illness. MK gene expression has been detected at many sites in healthy adults, including the gastrointestinal tract, kidneys, spleen, lungs, and the thyroid [7]. Many studies have demonstrated striking MK overexpression in patients with various pathologies, such as ischemia, inflammation, autoimmunity, and many cancers, compared with those in healthy controls [8]. MK has several functions, including cell proliferation, migration, survival, differentiation, and angiogenesis [9].

The authors report that MK expression can differ in women with PE compared with those in a normal pregnancy.

Materials and Methods

This prospective randomized controlled study was conducted on normotensive healthy pregnant women and pregnant women with PE at Bülent Ecevit University Research and Application Hospital, Obstetrics and Gynecology Department. Patients who had systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, and whose 24-hour urine contained ≥ 300 mg protein were included in the PE group. Blood pressure was taken using an appropriate cuff 1.5-fold the length of the upper arm circumference following a ten-minute rest in a seated position. The patient was warned not to smoke or drink coffee 30 minutes prior to their blood pressure measurement. Patients with a history of multiple pregnancies, preterm premature rupture of membranes, kidney disease (Cr upper limit of 1.4 mg/dl) or chronic systemic diseases, fetal gross or chromosomal abnormalities, gestational diabetes mellitus (DM) or pre-pregnancy DM, or a urinary tract infection were excluded from the PE group. The patients' instant urine tests and arterial blood pressure measurements were completed, and their 24-hour urine was collected. Those with protein levels < 300 mg, systolic blood pressure < 140 mmHg, and diastolic blood pressure < 90 mmHg were defined as the control group. All participants were selected from patients whose treatment and follow-up were conducted at the present hospital beginning the first week of their pregnancies.

The study group comprised of 45 pregnant women with PE and 35 normotensive healthy pregnant women as the control group. Women with PE who had a diagnosis of PE before gestational week 34 were included in the early PE group (n = 20). Women diagnosed with PE after gestational week 34 were in the late PE group (n = 45). This study was approved by Bülent Ecevit University Research and Application Hospital Ethics Com-

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Table 1. — *Median levels of MK in normal pregnancy, early PE, and late PE.*

Group	n	Midkine concentration (pg/ml)		
		Median	Mean	Std. deviation
Normal pregnancy	35	268	327.7	131.7
Early preeclampsia	20	235	791.4	1247.2
Late preeclampsia	25	224	272.9	232.3
Total	80	246	426.5	665.9

Table 2. — *MK levels in normal pregnancy and early PE groups.*

Group	N	Midkine concentration (pg/ml) Median	*p* value
Normal pregnancy	35	268	0.056
Early preeclampsia	20	235	

Table 3. — *MK levels in early PE and late PE groups.*

Group	N	Midkine concentration (pg/ml) Median	*p* value
Early preeclampsia	20	235	0.252
Late preeclampsia	25	224	

Table 4. — *MK levels in normal pregnancy and late PE groups.*

Group	N	Midkine concentration (pg/ml) Median	*p* value
Normal pregnancy	35	268	< 0.05
Late preeclampsia	25	224	

mittee.

Serum MK levels were measured in all subjects using the OmniKine Human Midkine enzyme-linked immunosorbent assay (ELISA) kit. The assay is based on a sandwich ELISA technique to quantify human MK in samples. Standards, controls, and samples were added to wells precoated with a human MK monoclonal antibody and incubated for two hours at room temperature. The plate was washed and biotinylated antibody was added to form an immune complex. Following a two-hour incubation at room temperature, unbound molecules were removed by washing, and streptavidin-HRP was added to the wells and incubated for 30 minutes. After washing, the substrate was added, and the color of the liquid changed to blue. The colored product is formed in proportion to the amount of MK present in the sample. The reaction was terminated by adding a stop solution, and the absorbance was measured using a microplate reader at a wavelength of 450 nm. The MK concentration in the sample was determined by comparing the optical density value of the sample to that in the standard curve.

SPSS 19.0 software was used for the statistical analysis. Descriptive statistics of the qualitative variables are given as frequencies and percentages, and quantitative variables are given as the mean standard deviation, median, and range values. The Shapiro–Wilk test used to test for normality, and the Mann–Whitney *U*-test was used for non-normally distributed variables. A p value < 0.05 was considered significant.

Results

No differences were observed for age or body mass index between the PE and control groups. Of the 80 pregnant women, 20 (25%) had early PE, 25 (31.2%) had late PE, and 35 (43.7%) were normotensive (Table 1). MK level was not different between normotensive women and those with early PE (Table 2). MK level was also not different between the early and late PE groups (Table 3). MK levels were significantly different between the normal pregnancy and late PE groups (Table 4; $p < 0.05$). The MK level decreased in the late PE group (median, 224–26 pg/ml) compared with that in the normal pregnancy group (Table 4).

Discussion

MK is a cytokine that is highly expressed in the mid-gestational period during embryogenesis [10]. It is a multifunctional pleiotropic growth factor and has antiapoptotic, migration promoting, angiogenic, antimicrobial, and other activities [11]. Hypoperfusion, hypoxia, and ischemia are critical factors in women with PE because a hypoperfused, ischemic placenta secretes a variety of factors into the maternal blood stream. These factors alter maternal endothelial cell function and lead to characteristic systemic signs and symptoms of PE [12-18]. Abnormalities in the development of placental vasculature during early pregnancy lead to a release of antiangiogenic factors, such as soluble fms-like tyrosine kinase 1 (sFlt-1). Increased placental expression and secretion of sFlt-1 appear to play a central role in the pathogenesis of PE. sFlt-1 antagonizes the proangiogenic biologic activity of circulating proangiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor, by binding to them and preventing interaction with their endogenous receptors, and alters maternal systemic endothelial function to cause hypertension and other disease manifestations [19]. Weckbach *et al.* showed the role of MK in hypoxia-induced angiogenesis. Angiogenesis was stimulated by MK, using VEGF as the positive control and phosphate buffered saline as the negative control [19]. However, the molecular basis for abnormal placental development and placental dysregulation of these pathogenic factors remains unknown. The role of angiogenic proteins during early placental vascular development are under investigation. One of the most significant biological functions of MK in adults is to preserve tissue viability under hypoxic stress, including that induced during ischemia [20]. Reynaud *et al.* also reported that MK is regulated by hypoxia via hypoxia-inducible factor-1α (HIF-1α) [12]. HIF-1α is expressed in the early human placenta and is a key mediator of hypoxic and inflammatory responses. HIF-1α regulates the expression of many growth factors, including VEGF and its receptors, which are implicated in development of the placental vasculature [13]. Increased sensitivity to angiotensin II has been reported in

women with PE [21]. MK plays a role in regulating blood pressure via the renin-angiotensin system (RAS) [22, 23]. One of the first links between MK and the RAS was made by investigating the levels of the RAS components in MK+/+ versus MK −/− mice aortas. The first observations showed that expression of renin, angiotensinogen, and Ang II type 1 and type 2 receptor mRNAs was increased, and that angiotensin-converting enzyme levels were decreased in MK-deficient mice [22].

In recent years, many studies have provided data on MK. MK is affected by hypoxia and affects systemic endothelial functions, the RAS system, and angiogenesis [24-26], and these characteristics play roles in PE pathophysiology. Thus, the present authors expected serum levels of MK in women with PE to differ from those in normotensive pregnant women. The present observations show that plasma MK levels were decreased significantly in the late PE group compared with the normal pregnancy group. The physiology of pregnancy, unknown knowledge about the molecular basis for PE pathogenesis, and the diverse roles of MK in the humans may have influenced the MK levels in the PE group. Notably, the small sample size was a major limitation of this study. This is the first study to investigate MK in normally pregnant and PE women. More prospective studies using a larger sample size are desirable to confirm these findings.

Conclusion

This is the first study to investigate differences in MK levels between normally pregnant and early and late PE women. The present findings demonstrated a significant decrease in serum MK levels in late PE women compared with normally pregnant women.

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