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Serum Macrophage Migration Inhibitory Factor in the Prediction of Preterm Delivery

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Abstract

Serum Macrophage Migration Inhibitory Factor in the Prediction of Preterm Delivery.

Objective—Macrophage migration inhibitory factor (MIF) is a soluble mediator that helps govern the interaction between cytokines and stress hormones (e.g. cortisol). We determined if maternal MIF levels predicted subsequent preterm delivery (PTD).

Study Design—A nested case-control study measuring serum MIF concentration at 9–23 weeks gestation in women who ultimately delivered preterm (n=60) compared to control women who delivered at term (n=122). We also examined the connection of MIF with self-reported psychosocial variables.

Results—MIF was elevated in the PTD cases (p=0.0004), and log MIF concentration showed a graded response relationship with likelihood of PTD. High MIF was also associated with maternal risk-taking behavior, which itself was a risk factor for PTD. MIF remained associated independently with PTD after adjusting regression models for several other PTD risk factors (OR, 3.11, 95% CI 1.54–6.30).

Conclusion—High serum MIF concentration in early to mid-pregnancy is linked with subsequent preterm delivery.

Keywords

Cytokine; Macrophage Migration Inhibitory Factor; Pregnancy; Preterm Birth; Cortisol

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INTRODUCTION

Pathogenic mechanisms of preterm labor and delivery can entail diverse antecedents (infections, behavioral stressors) that have been proposed to share some common molecular pathways. Numerous pathways have been implicated, but those involving proinflammatory cytokines and stress-hormones (e.g. cortisol, corticotropin releasing hormone) have come to the forefront because they are stimulated by both infections and psychological stress or depression. Cortisol is an endogenous glucocorticoid that suppresses proinflammatory cytokine production by acting on glucocorticoid receptors, which have a broad tissue distribution including immune cells, brain, and female reproductive organs (uterus, cervix, placenta).

Macrophage migration inhibitory factor (MIF) is a cytokine that has been well demonstrated to override the action of glucocorticoids at target tissues, hence amplifying proinflammatory responses. MIF is expressed in the human ovary, endometrial cells, 1st trimester trophoblasts, reflected fetal membranes, and chorioamniotic membranes at delivery. In addition, soluble MIF is present in amniotic fluid, maternal blood, umbilical cord blood, and the intervillous circulation of the placenta. Consistent in its role as mediator of innate immunity and inflammation, MIF is induced in various infections, including those that occur during pregnancy.

There is a lack of data in humans on the alteration of MIF in psychological stress or other behavioral risk factors for preterm delivery. Studies in vitro and experimental animals suggest that MIF secretion is induced by psychological stress, and the stress hormone, corticotropin releasing hormone (CRH). Thus MIF is linked with infections, reproductive function, and psychological stress, but the details of how MIF may participate in PTD are lacking. The primary objective of this study was to determine if maternal peripheral blood levels of MIF, measured at 9–23 weeks gestation, correlate with subsequent non-induced preterm delivery. We further examined the relationship between MIF levels and self-reported psychological variables. Considering that bacterial vaginosis (BV) is a risk factor for preterm delivery, we also examined the interrelationship between BV, MIF, and preterm delivery.

MATERIALS AND METHODS

We performed a nested case-control study to determine if serum MIF in early to mid-pregnancy was associated with subsequent preterm delivery. The study cohort has been described previously. Briefly, all women receiving prenatal care at the Department of Obstetrics and Gynecology at Odense University Hospital, Denmark, between November 1992 and February 1994 (n = 3596) were invited to participate in the study. Women who enrolled (n = 3174; 88.3%) did so at their first antenatal hospital visit, before 24 full gestational weeks, and 2927 (81.4%) completed all test and questionnaire requirements. The criteria for exclusion were insufficient responses to the questionnaires, placental previa (diagnosed after 30 full gestational weeks), history of severe fetal congenital malformations in previous pregnancy, and uterine cervix insufficiency treated with cervical cerclage. All enrolled participants gave written informed consent that met the requirements of the scientific ethics committee for Vejle and Funen counties, Denmark, and the institutional review board for research on human subjects, Centers for Disease Control and Prevention (Atlanta, GA). Data collection methods were approved by the Danish Data Surveillance Authority.

A pelvic examination including clinical observations was performed on each participant upon enrollment. An early blood sample was taken for serum assays at this visit. Accordingly, this sample was taken before it was known which women would deliver preterm.
Results of ultrasonographic measurements of the biparietal diameter and the femur length of the fetus at the 18th week gestation confirmed gestational age and the estimated date of delivery for 97.5% of the participants. The obstetrical medical record was supplemented with data from questionnaires completed by the participant concerning maternal behaviors, psychosocial stress, socioeconomic factors, work demands, and prior obstetrical problems. The social stress questionnaire asked the women whether they were experiencing psychological stress due to quarrels, violence, or financial difficulties. Risk-taking behavior was defined as seldom or no use of seatbelts while driving. We used seat-belt usage as an measure of risk taking behavior because it has been reported to correlate with some other indices of this behavior (taking dangerous dares and bicycle helmet use) 26, and we reasoned it may present fewer obstetric confounds than other commonly-used indices of risk-taking behavior such as sexual promiscuity and use of birth control. The lack of basic education (less than or equal to 9th grade) was used as a proxy variable for low SES. 24

Study groups

Idiopathic preterm delivery cases—From the study base of 2927 pregnant women who delivered, 170 had a preterm delivery (<37 full gestational weeks) and were classified as cases, of which 58 were medically indicated (caesarean section and induction of labor due to obstetric complications, e.g., preeclampsia and fetal distress) and the remaining 112 were idiopathic. Of the 112 preterm deliveries, ten were fetus mors imminens or had precipitous delivery, leaving 102 women in the preterm study group. Eighteen women had multiple gestation preterm delivery and 84 women had singleton preterm delivery, of which 24 women were treated with corticosteroids during labor and thus excluded from analyses leaving 60 preterm deliveries for analyses in this study.

Term delivery controls—A subset of 122 women in the cohort who delivered at term (38–43 weeks gestation) served as the term delivery comparison group (controls). Individual controls were selected based on the day of their 2nd routine prenatal visit. The trigger for selecting a particular woman as a control was that she had a prenatal appointment (and blood draw) at a gestational age that was comparable to the gestational age at delivery of a preterm case. 24, 25 While this gestational age-matching criterion was invoked for other studies of the cohort that were concerned with markers and risk factors arising around the time of preterm delivery, 24 such matching is believed to be independent of the characteristics measured at the 1st antenatal visit which is the focus of the current study. Therefore, for analysis purposes, the data were treated as an unmatched case-control study. All controls also met the inclusion criteria of having a blood sample taken at the first prenatal visit (prior to 24 weeks gestation), allowing case-control comparison for MIF as an early blood biomarker of subsequent delivery status.

Serum analysis

Serum MIF was assayed by sandwich ELISA according to the manufacturer’s’ protocol using a mouse monoclonal anti-human MIF capture antibody, and a horseradish-peroxidase (HRP)-conjugated anti-human MIF polyclonal detection antibody (R and D Systems, Minneapolis, MN). Each run included a standard curve, and runs were counterbalanced to include a combination of both cases and controls. The inter-assay quality controls included normal serum as well as high and low MIF disease-state samples (The Biotech Source, Windham, Maine; Complex Pharma, Ontario, Canada) yielding a median coefficient of variation of 17.6%. The intra-assay CV was 6.6%.
Statistics

MIF levels in cases and controls were compared with a two-tailed t-test after values were normalized by log transformation. The normalized MIF was further categorized in 5 equally spaced categories and analyzed in a logistic regression to test for trend. When it was established that the risk of PTD was steadily increasing with MIF level, the normalized MIF was analyzed in a logistic regression on the continuous scale. Serum MIF concentration was dichotomized through a ROC analysis optimizing the predictive effect of MIF on PTD. Logistic regression models used this cutoff and were fitted yielding odds ratios as effect measures, stated with 95%-confidence intervals (CI).

Models took into account putative confounders of the association between MIF and PTD, as well as effect modification. Variables were selected based on reported association with PTD in the literature, and sufficient frequency of the exposure in our sample (e.g. preeclampsia and non-Caucasian race were not included as factors because they were rare in our sample). Accordingly, models were adjusted for tobacco smoking, previous preterm delivery, lower socioeconomic status (SES), serious maternal medical condition, nullipara, high BMI, and maternal age. Analyses were carried out using Stata 9.2 (StataCorp LP, 4905 Lakeway Drive, College Station, Texas 77845, USA).

RESULTS

Characteristics of subjects are shown in table 1. Women who ultimately delivered preterm had their 1st prenatal visit at a later gestational age than control women, but our graphical and correlation analysis found no evidence a relationship between the gestational age at the 1st visit and serum MIF levels (data not shown). The median MIF concentration was significantly (p<0.001) higher in the PTD cases (Median=9.22 ng/ml, IQR 6.22–12.06) compared to controls delivering at term (Median=7.00 ng/ml, IQR=5.64–9.17).

The role of MIF as a predictive factor in PTD was further examined using logistic regression. Log MIF showed a clear dose response relationship with log odds for PTD (figure 1). On a continuous scale, the odds ratio for PTD was 4.16 (95% confidence interval, 1.83–9.48, p=0.001) signifying that for each natural log increase of 1 unit, the odds of PTD increased by 4.16 fold. We constructed a ROC curve to further assess the sensitivity and specificity of MIF levels in predicting subsequent PTD. Based on this curve (figure 2), the optimal cutoff for MIF was 9.16 ng/ml (representing the 75th percentile for controls). The sensitivity of MIF in predicting subsequent preterm delivery was 53.3% (CI 46.1–60.6), and the specificity was 75.4% (CI 69.1–81.7). The cutoff of 9.16ng/ml for MIF concentration was used in all subsequent logistic regression models.

Women in the high MIF category were 3.5 times more likely to deliver preterm than women in the low category; unadjusted odds ratio 3.50, CI 1.82–6.74, p<0.0005. The relationship between MIF and PTD remained significant after controlling for tobacco smoking, previous preterm delivery, lower socioeconomic status (SES), serious maternal medical condition, nullipara, BMI >30, and maternal age >35 (adjusted OR=3.33, CI 1.66–6.67).

MIF and psychological variables

Table 2 shows the relationship of self-reported behavioral variables with MIF and PTD. The positive association between social stress and MIF (OR 3.04) failed to reach statistical-significance, and the risk for PTD was not increased as a function of this perceived stress. In contrast, women with risk-taking behavior were more likely to have elevated MIF (unadjusted OR 6.92). Moreover, this behavioral measure was also a significant risk factor for PTD.
Despite this relationship between risk-taking behavior, MIF, and PTD, in multivariate analysis there was an independent contribution of elevated serum MIF to the likelihood of preterm delivery after adjusting for risk-taking behavior in addition to the other potential confounders (table 2).

**Infection and bacterial vaginosis**

Elevated MIF was not associated with diagnosis of BV at the first antenatal visit, OR=1.10, CI 0.39–3.11. Likewise, BV was not significantly-associated with preterm delivery, OR 0.76, CI 0.33–1.76. However, BV could still be influencing the relationship between MIF and PTD outcome, and our models suggested this possibility. Therefore, we explored this relationship further by stratifying on BV diagnosis. In the subset of women without a BV diagnosis, the link between high MIF levels and PTD remained significant, OR=2.78 CI 1.36–5.69. For women who had a BV diagnosis, the link between high MIF and PTD was also significant, OR= 22.67, CI 2.32-221. Thus, in both BV strata, high MIF predicted PTD. While the data suggested this relationship was especially strong in women with BV, we did not have sufficient power to distinguish the two strata from each other (interaction term, 2.70, CI 0.21–34.17).

**COMMENT**

In this study elevated MIF levels in maternal serum, showed a highly significant association with future preterm delivery. There are few prior studies of MIF in preterm labor and delivery, and none have examined early timepoints in gestation and adjusted analysis for confounders as in the current study. Still, as a single predictor of PTD, MIF showed inadequate specificity and sensitivity to have immediate applicability in clinical practice.

Two prior studies of MIF in preeclampsia came to discrepant conclusions, though one study suggested that MIF levels at delivery showed the most pronounced increases in a subgroup of preeclampsia patients who delivered prior to 34 weeks gestation, suggesting (in light of the current findings) that MIF may be elevated in diverse causes of PTD. One way to examine this would be to measure MIF in the members of this cohort who had medically-induced preterm deliveries.

CRH has been shown to stimulate MIF, raising the question of whether the progressively-rising CRH levels that occur with advancing gestation are accompanied by rising MIF levels. There are conflicting data in the literature concerning the regulation of MIF during pregnancy and normal labor but serial measurements of circulating maternal MIF indicate that changes in this cytokine are subtle, and do not mirror the exponential rise of CRH levels.

MIF is well positioned physiologically to influence behavior because it is present in the brain and it has been shown to modulate catecholamine metabolism and neuronal activity in vitro. We found that women with risk-taking behavior were more likely to manifest high MIF compared to those without this behavioral factor, and (as previously reported for this cohort) risk-taking behavior (seat belt usage) was connected to an increased rate of PTD. We considered that overproduction of MIF may constitute the biochemical link between this behavior and preterm delivery, but our adjusted logistic regression models did not support this idea. There are numerous intermediate or co-varying factors that could plausibly account for the connection between seat belt usage and PTD (e.g. substance abuse, higher exposure to sexually transmitted diseases, inadequate self care or utilization of prenatal services, more unplanned pregnancies). The relationship between these factors and MIF has not been determined.
In a recent study of amniotic fluid collected between 19 and 34.6 weeks gestation, women with intra-amniotic infections had higher MIF than women with sterile amniotic fluid. Among women with preterm labor, high amniotic fluid MIF was associated with a shorter amniocentesis-to-delivery interval suggesting a relationship between MIF and preterm delivery.

Polymorphisms in the human MIF gene have been linked to overproduction of MIF and the presence or severity of various inflammatory disorders. Because MIF is known to be is stimulated by bacterial infections, the link we found between high MIF and subsequent PTD could in theory be driven by infection in women predisposed toward exaggerated MIF responses. Nevertheless, our data showed that MIF was significantly-associated with PTD in women with and without bacterial vaginosis diagnosis.

MIF upregulates the expression of the TLR4 toll-like receptor (the recognition molecule for bacterial endotoxin) and induces cyclooxygenase-2. Moreover the putative cellular receptor for MIF is CD74, an immune molecule that is upregulated in fetal membranes of patients with PTD associated with preterm premature rupture of membranes pPROM. Thus there are multiple and perhaps overlapping pathways by which MIF could facilitate inflammation during labor.

There are important limitations of this study. Our findings from a population of mostly healthy Caucasian women may not extrapolate well to more mixed ethnic populations with less access to healthcare and higher rates of PTD. Moreover, our focus on women with late PTD who lacked definitive clinical antecedents (i.e., idiopathic PTD) limits our conclusions on the role of MIF in deliveries that occur earlier (before 30 weeks gestation) or have unambiguous causes. Selection bias can hardly explain the findings since the participation rate was high and nonparticipation or loss to follow-up took place prior to the collection of exposure and outcome measures. A strength of the study is that MIF was detectable in all subjects and showed a concentration-dependent relationship with future PTD. Given the large number of anti-MIF compounds in development for other disorders, therapy directed at MIF or guided by MIF concentrations could have potential for PTD intervention.

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REFERENCES


Figure 1.
Odds of PTD on a log scale against MIF in categories equidistant on the log scale, ticks indicate the center of each interval.
Figure 2.
The area under the ROC curve for MIF (0.65, CI 0.57–0.74) was significantly greater than the reference area (0.5), solid line.
Table 1

Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Term</th>
<th>Preterm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>122</td>
<td>60</td>
</tr>
<tr>
<td>Previous preterm delivery ‡</td>
<td>6 (4.9%)</td>
<td>11 (18.3%)</td>
</tr>
<tr>
<td>Nullipara</td>
<td>61 (49.2%)</td>
<td>30 (50.0%)</td>
</tr>
<tr>
<td>Smoking during pregnancy</td>
<td>12 (9.8%)</td>
<td>4 (6.7%)</td>
</tr>
<tr>
<td>Serious medical disease</td>
<td>5 (4.1%)</td>
<td>5 (8.3%)</td>
</tr>
<tr>
<td>BV at 1st visit</td>
<td>23 (18.9%)</td>
<td>9 (15.0%)</td>
</tr>
<tr>
<td>Median Body Mass index (BMI) *</td>
<td>21.6 (15.8, 36.1)</td>
<td>21.9 (17, 34.4)</td>
</tr>
<tr>
<td>BMI&gt;30</td>
<td>6 (4.9%)</td>
<td>5 (8.3%)</td>
</tr>
<tr>
<td>Maternal age &gt;= 36 years</td>
<td>7 (5.7%)</td>
<td>4 (6.7%)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>36 (29.5%)</td>
<td>17 (28.3%)</td>
</tr>
<tr>
<td>Low SES</td>
<td>3 (2.5%)</td>
<td>6 (10.0%)</td>
</tr>
<tr>
<td>Risk behavior ‡</td>
<td>6 (4.9%)</td>
<td>9 (15.0%)</td>
</tr>
<tr>
<td>Social stress</td>
<td>13 (10.7%)</td>
<td>10 (16.7%)</td>
</tr>
<tr>
<td>GA at delivery *‡</td>
<td>40.4 (37.3, 43.3)</td>
<td>35.8 (29.9, 36.9)</td>
</tr>
<tr>
<td>GA age at 1st visit *</td>
<td>16.0 (9.0, 23.0)</td>
<td>18.4 (9.3, 21.9)</td>
</tr>
<tr>
<td>Maternal age at delivery ‡</td>
<td>29.2 (20.0, 40.1)</td>
<td>27.4 (18.7, 41.9)</td>
</tr>
<tr>
<td>Log serum MIF at visit 1 ‡</td>
<td>1.97 (0.39)</td>
<td>2.20 (0.41)</td>
</tr>
<tr>
<td>Birth Weight (g) ‡</td>
<td>3578.2 (574.5)</td>
<td>2632.7 (492.3)</td>
</tr>
</tbody>
</table>

Percentages are within outcome category

* median (range)

† mean (standard deviation)

‡ p<0.05 for case-control comparison (tests comparing medians were continuity corrected). Smoking=10 cigarettes or more per day; BV, Bacterial Vaginosis; BMI, Body Mass Index; SES, Socioeconomic Status using lack of basic education as a proxy; Risk-taking behavior as indicated by lack of seat-belt usage; GA, Gestational Age; MIF, Macrophage migration Inhibitory Factor
### Table 2
Association of MIF and PTD with Maternal Behavior

<table>
<thead>
<tr>
<th>Factor</th>
<th>Unadjusted odds ratio (95% CI)</th>
<th>PTD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High MIF</td>
<td>1.68 (0.69–4.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.41 (1.15–10.09)</td>
</tr>
<tr>
<td></td>
<td>Adjusted odds ratio†</td>
<td>3.11 (1.54–6.30)</td>
</tr>
<tr>
<td>Social Stress</td>
<td>3.04 (0.93–9.89)</td>
<td></td>
</tr>
<tr>
<td>Risk-taking behavior</td>
<td>6.92 (1.20–39.94)</td>
<td></td>
</tr>
<tr>
<td>MIF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Lack of seat belt usage
† Association between MIF and PTD outcome adjusted for risk-taking behavior, tobacco smoking, previous PTD, lower SES, serious maternal medical illness, nullipara, body mass index (>30), and maternal age (>35 years).