Expression of matrix metalloproteinase-9 (MMP-9) in human midpregnancy amniotic fluid and risk of preterm labor

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Summary

Object: This work stands as a pilot study in assessing the reliability of metalloproteinase-9 (MMP-9) as a marker for intraamiotic infection and preterm birth already in early pregnancy. *Subject:* 100 amniotic fluids taken at the Midwife Obstetrics and Gynaecological Clinic of the University of L'Aquila (Italy). *Results:* Our results show that MMP-9 is a sensitive marker of intraamionic infection (an important risk factor for preterm delivery) already in early pregnancy, because only women with a significant elevation were subsequently exposed to preterm birth. *Conclusions:* Early identification of women at risk of preterm birth is of important clinical significance. In- deed exposing women to deep diagnostic and therapeutic protocols could possibly reduce the incidence of preterm birth in the near future and have a positive impact on fetal prognosis related to unknown intraamniotic infection.

Key words: Preterm birth; Metalloproteinases; Intraamiotic infections.

Introduction

Remodeling of the extracellular matrix is a fundamental event in both physiological and pathological conditions [1]. The metalloproteinase array (MMPs) are a family of enzymes involved in many physiological and pathological processes that affect the extracellular matrix. The activity of MMPs in the extracellular space is strictly controlled by endogenous inhibitors (TIMPs) [2, 3].

The balance between gelatinase and inhibitors is crucial in the evolution of pregnancy, and an alteration of this balance underlies physiological rupture of the membranes during labour and obstetrical complications such as PROM and preterm birth [4].

A condition of subintrauterine/fetal inflammation during early pregnancy can be the basis for subsequent preterm birth, since the physiopathological processes that contribute to preterm rupture of membranes and /or to preterm birth may already be triggered in the first/second trimester of pregnancy.

Numerous studies state the role of MMP-9 in the mechanisms responsible for term or preterm breaking of the amniotic membrane and stress the importance of microbial infection of the amniotic cavity for the production and release of MMP-9, which was thus confirmed as a sensitive and specific marker in the case of sub-corioamnionitis and prediction of preterm birth [5, 6].

Given this, the purpose of this work was to analyze MMP-2 and MMP-9 in the amniotic fluid of women who had undergone amniocentesis at 17 weeks of gestation and in others that were instead subjected to caesarean, to confirm the literature data about the expression in those gestational stages and to relate these results with the evolution of pregnancy and or specific complications.

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Materials and Methods

One hundred samples of amniotic fluid (marked with a serial number from 1 to 100) were taken at the Gynaecology and Obstetrics Clinic of the University de L'Aquila between October 2005 and February 2006. The samples of amniotic fluids were donated by randomly selected patients with their explicit and informed consent. Amniotic fluids were all taken with transabdominal amniocentesis between 16 + 5 and 18 + 4 weeks of pregnancy, except for fluids 60, 81, 82, 83 and 100 which belonged to patients undergoing caesarean in a period between 28 +5 and 41 +1 weeks of pregnancy.

Patients whose amniotic fluid was taken by amniocentesis at 17 weeks were contacted again after the expected time of delivery to gather news about the subsequent evolution of the pregnancy. For determination of metalloproteinases MMP-2 and MMP-9 (Laboratory of Biochemistry and Molecular Biology of the University of L'Aquila) the zymogram technique was used; this procedure requires the preparation of a polyacrylamide gel with subsequent revelation of gelatinase activities.

For protein balance an indirect method was used - comparison of the protein content of samples with one of a standard protein (bovine serum albumin -BSA- at a concentration of 1 mg/ml).

We used the method proposed by Bradford [7] in 1976, which requires a reactive BIO-RAD where proteins bind to a dye (Coomassie Blue).

The acrylamide-bisacrylamide gel (40% w/v) was prepared with an anionic detergent, sodium-dodecyl sulfate (SDS), according to the method reported by Laemmli [8].

Results

Obstetric complications in the third trimester developed in our sample. Specific conditions are listed in Table 1. Figure 1 shows zymography analysis results.

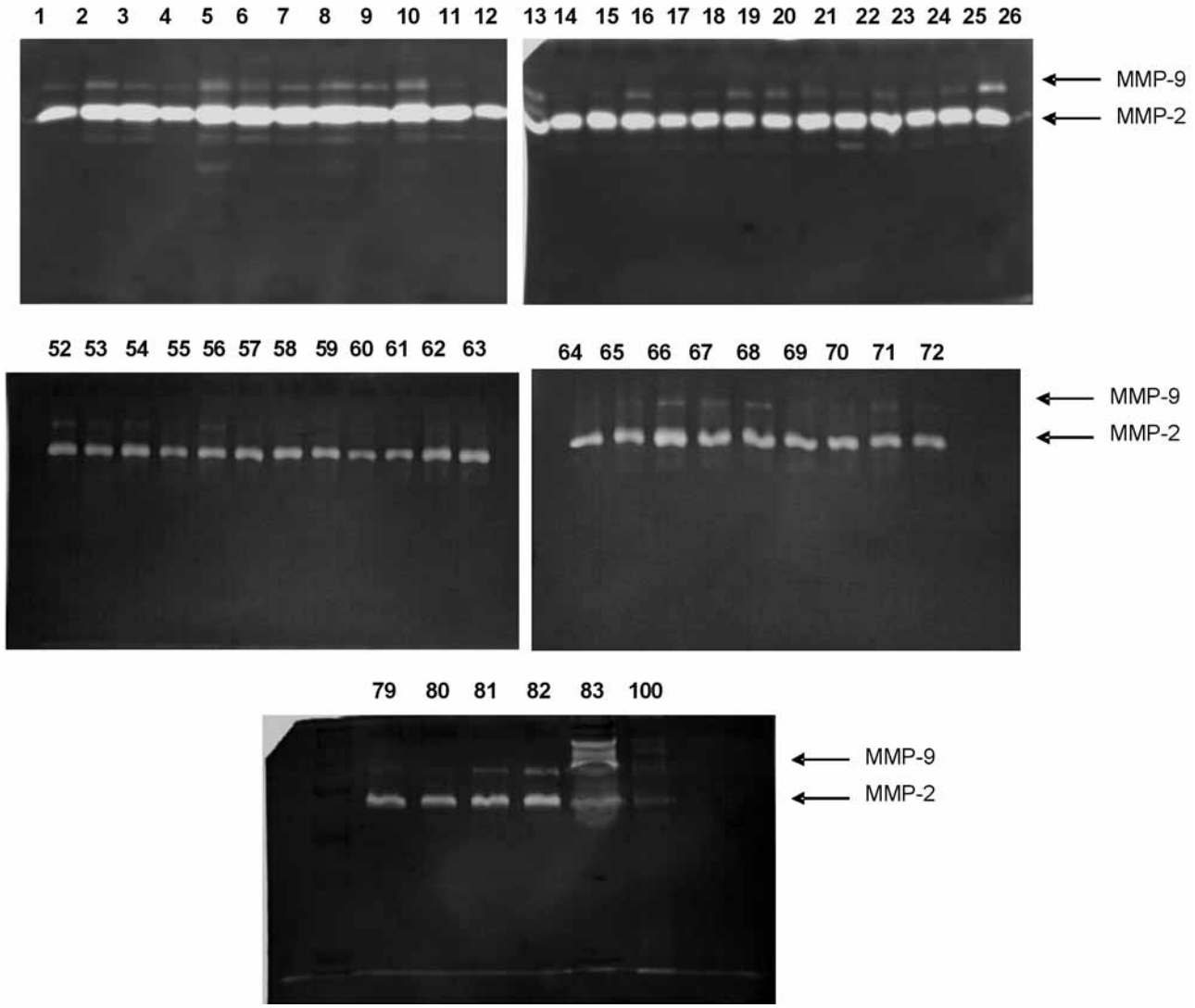

Figure 1. — Most representive expressions of MMPs in amniotic fluid.

Table 1. — *Pregnancy complications in our sample*.

Complication	No. of cases	ID number
Preterm birth	6	5, 10,16, 17, 26, 83
pPROM	2	16, 26
PROM	5	9, 34, 54, 81
Gestational hypertension	2	17, 31
Sepsis during pregnancy	1	83
Urogenital tract infections	11	5, 7, 9, 10, 20, 26, 30, 59, 60, 72, 82

The activity of MMP-2 was uniformly expressed in all samples, while the activity of MMP-9 showed variability.

The nearly constant expression of MMP-2 perfectly reflects the physiological expression of the enzyme, representing the most abundant gelatinase in the amniotic fluid from the second trimester of pregnancy and remaining relatively stable up to increase when labor starts [9, 10].

Figure 9.1 shows that MMP-9 was expressed, albeit at low levels, in almost all samples but shows a significant increase of its activities in the fluid 5.8, 10, 16, 26, 81 and 82. The expression of MMP-9 instead had an intermediate value between the standard of fluid analysed and those just mentioned, in samples 7, 9.

Physiologically the expression of MMP-9 in the amniotic fluid was minimal through the whole gestation period and underwent a significant increase around the first two days of labor, which helps in triggering contractile activities and breaking the membranes. Analysis of the zymography also confirms this fact (see increased expression in 81 and 82 from caesareans with contractile activity and low contractile activity in 60 and 100 from caesareans without any activity).

MMP-9 plays a key role not just during physiological delivery, but also in conditions such as PROM, pPROM, endoamniotic infections and preterm birth [9, 10].

Checking the medical history of patients in whom gelatinase activity (MMP-9) is more expressed has shown that they are linked to a single theme: each had a bacterial infection during pregnancy and, more precisely, during the first trimester:

– Patients of samples 5 and 26 developed vaginitis by *Gardnerella vaginalis*;

– The patient of sample 10 developed asymptomatic bacteriuria;

– The patient of sample 16 reported at 15 weeks positivity of a vaginal swab for beta haemolytic streptococcus (group B);

– The patient of sample 8 voluntary interrupted the pregnancy for therapeutic reasons - *Cytomegalovirus* first infection - the results of PCR on amniotic fluid confirmed the presence of viral RNA;

– The patient of sample 82 showed infection from *E. coli* and *Ureaplasma urealyticum*.

The pregnancy of patients 5, 10, 16 and 26 were complicated with preterm birth between 33 and 36 gestational weeks.

We also noted intermediate gelatinase activity MMP-9 in samples 7 and 9. Both patients belonged to the group of women who contracted urogenital infections during the first quarter but their pregnancies concluded with spontaneous birth in the long term, albeit with PROM (39 weeks) in patient 9.

Discussion

The role of urogenital infections in induction of preterm birth is now clear. Among most significant urogenital infections we want to highlight the role of bacterial vaginosis in the first quarter, which is in fact responsible for 21.9% of preterm births and 43.8% of PROM [11, 12].

In the observed sample patients 5 and 26 reported a diagnosis of *bacterial vaginosis* in the 1st trimester. For bacterial vaginosis there is a significant association of risk factors in the determination of preterm birth [13]. Significant in this regard is low socio-economic level, poor prenatal care and the state of chronic hypothyroidism in patient 5 and smoking during pregnancy and fetal death in the previous patient 26. Also confirming the literature data, patient 5 had early interrupted treatment with oral clindamycin and patient 26 had followed topical therapy with the same drug which does not significantly reduce the risk of preterm birth compared to oral therapy [14].

Patient 10 reported history of recurrent pre-pregnancy urinary infections, asymptomatic bacteriuria in urine culture tests during the first obstetric visit, genital blood loss in the first trimester and development of a pyelonephritis during the third trimester. Recently, the role of asymptomatic bacteriuria as a risk factor for preterm birth has been reevaluated by many authors who associate this condition especially with the risk of a subsequent pyelonephrite [15].

Patient 16, who at 15 weeks had a positive vaginal swab for beta haemolytic streptococcus (group B), was hospitalised at 36 weeks for pPROM and threat of preterm birth that led to spontaneous delivery in the same day [16]. Currently, the role of that etiologic agent in the onset of preterm birth has been quite reduced even though it is found in the vaginal secretions of women who give birth at term - much more rarely than those who give birth prematurely. As for the patient in question, we should point out the simultaneous presence of other risk factors such as black race, smoking during pregnancy and low socio-economic level.

Conclusions

The aim of this work was to confirm the reliability of MMP-9 as a marker for preterm birth and intraamniotic infection, even in early periods of pregnancy. The methodology was mainly based on the possibility of finding markers that could identify an early state of endouterine infection; development of such markers could certainly derive important therapeutic implications. Although results certainly seem encouraging, they must be further studied with the analysis of a larger sample and a quantitative analysis, and not just qualitative expression of the enzyme. The idea stems from the study of recent literature which suggests that at-term pregnancies complicated by corioamnionitis and PROM show important collagenolytic activity (MMP-9), usually absent in normal at-term pregnancies [17]. Since among the different metalloproteinases MMP-9 is the one whose production and release is inducible by certain conditions, and especially by endoamniotic infections, it was investigated and confirmed as a marker in the case of subclinical endo amnionitis and prediction of preterm birth.

The dosage in such conditions reaches values of sensitivity, specificity, positive predictive value and negative predictive value respectively, 83%, 100%, 100% and 90%.

The fact that enzyme quantification methods on the maternal serum or even on saliva (which reflects the expression at the level of amniotic fluid) are being developed will exceed the limit and the fair objection as to whether it is legal to practice amniocentesis, an invasive diagnostic technique, which could result in preterm childbirth [18].

An analysis of zymographies concluded that the only patients in whom MMP-9 expression showed a higher than normal expression were those who had urogenital infections during the first quarter, and that preterm birth occurred only in those where the enzyme expression was greater. In this regard we should point out that early and adequate (oral) therapy could reduce the risk of subsequent complications.

We would like to stress that enzyme expression at 17 weeks did not allow a precise time interval to be determined between enzyme dosage and the time of the subsequent preterm birth. It is not clear whether with a dose quantity of MMP-9 it would be possible.

Patients with subsequent preterm birth typically showed a combination of etiologic factors where proba-

bly only the endoamniotic infection, without such competition, would never have led to a subsequent preterm birth or PROM.

In conclusion early identification of women at risk of preterm birth certainly has important clinical significance. Indeed, submitting a woman to thorough diagnostic investigations and maybe treatment protocols could in the near future allow the incidence of preterm birth to be reduced, positively impacting on fetal prognosis related to unrecognised states of intraamniotic infection.

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