原　著

RELATIONSHIP OF METALLOTHIONEIN TO ZINC METABOLISM DURING PREGNANCY

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Abstract : The amounts of metallothionein (MT) and related substances in amniotic fluid and urine were measured, and the role of the MT contained in these fluids was studied.

Amniotic fluid without blood was collected during Caesarian sections. Total urine obtained during one day was collected from newborn infants (0-6 days), and spot urine was collected from pregnant women. Sera were also collected. MT in amniotic fluid and urine was measured by radioimmunoassay. In amniotic fluid, a positive relation was observed between MT and ß2-microgloblin (MG), but not between MT and zinc. In contrast, a positive relation was observed between MT and zinc in urine of newborn infants, but not between MT and MG. Serum zinc levels were significantly lower in postpartum women than healthy young women, and urinary zinc levels were markedly higher in newborn infants than healthy young women.

In conclusion, the level of MT in amniotic fluid may reflect fetal renal function, and another function of MT in the fetus and newborn infant is to supplement zinc in the body.

Key words : metallothionein, zinc, $ß_2$-microgloblin, amniotic fluid

INTRODUCTION

The physiological state of elements such as zinc, iron, and calcium affects the growth and development of the fetus, placenta, and maternal tissues during pregnancy[1,2]. The plasma zinc level decreases during pregnancy and the decline is related to the birth weight or the gestational age of the infant[3,4]. It was recently reported that zinc supplementation improved survival in low-birth-weight infants[5]. Therefore, an adequate dietary intake of zinc during pregnancy and infancy is recommended.

Metallothionein (MT) is an acute phase, low molecular mass, cysteine-rich protein. MT is produced in most organs in response to metals, organic solvents, ultraviolet irradiation, starvation and so on[6]. It is reported that MT functions in the detoxification of heavy metals, storage of essential metals like zinc, scavenging of free radicals, and cell proliferation[6-8].

During pregnancy, MT is stained immunohistochemically in various fetal tissues[9]. MT may have several functions during pregnancy.

Continuous intake of zinc is required during pregnancy, as zinc deficiency causes teratogenesis, growth retardation, prolonged parturition and a variety of developmental disorders in the offspring[4]. And MT acts to transfer zinc from the mother to the fetus[10,11]. In addition, zinc accumulates in fetal livers connected with hepatic MT synthesis[2]. Furthermore, zinc concentration in amniotic fluid is related to fetal weight[4] and MT may act to maintain zinc concentration in the amniotic fluid. In contrast, Goyer and Cherian[12] reported that MT in placenta acts as a barrier to maternal-fetal cadmium transfer using cadmium-treated rats, although a positive relationship between zinc and MT in placentas was found. It was also reported that MT acted in the detoxification of cadmium in amniotic fluid since the MT level in amniotic fluid reflected the influence of tobacco smoke [13]. Therefore, we measured $ß_2$-microgloblin (MG) and N-acetyl glutaminidase (NAG) activity in amniotic fluid for observation of renal dysfunction[14] and compared them with MT levels. The aim of the present study was to observe the role of MT in the fetus and newborn infant by examining amniotic fluid, serum and urine.

MATERIALS AND METHODS

The serum, amniotic fluid, and urine used in the present study were obtained with consent from women who consulted Kosaka Women's Hospital (Osaka, Japan) regarding pregnancy. Serum for control was obtained from female students (n=14, 18–22 years old). Pregnant women were divided into three groups : Early (n=22, 5–21 weeks), Middle (n=31, 22-28 weeks), and Late (n=24, 28–40 weeks), and compared with control, postpartum women (n=20, 2 days after parturition), and newborn infants (n=12, 0–6 days). Amniotic fluid without blood checked for albumin was collected during Caesarian section (n=12). One day urine in newborn infants and spot urine in control and pregnant women was used. Serum, urine, and amniotic fluids were stored at -20°C until the experiments.

MT levels were measured by radioimmunoassay (RIA), zinc levels were measured by induced coupled plasma atomic emission spectrometry (ICP-AES), and MG levels were measured by a turbidity immunoassay method. NAG activity in amniotic fluid and urinary creatinine levels were also measured using an automatic analytical machine for clinical chemistry. Urinary MT, zinc and MG levels are shown as creatinine levels.

Data show the mean ± standard error (SE). For statistical analysis, a one-way analysis of variance was used and significance was compared using the Student-Neuman-Keurs method.

RESULTS

Amniotic fluid with a low albumin level (<100 mg/L) was used to exclude the effects of blood contaminants in the present study. MT was detected in all samples at 38.3 ± 5.3 ng/ml, and MG was 2489.4 ± 216.9 ng/ml. By contrast, the zinc level was just 0.7 ± 0.1 μg/ml, and NAG activity was 8.3 ± 0.4 U/l. A positive relationship was observed between MT and MG ($r=0.682$, $p<0.01$) as shown in Fig. 1, but not between MT and zinc or between MT and NAG activity.

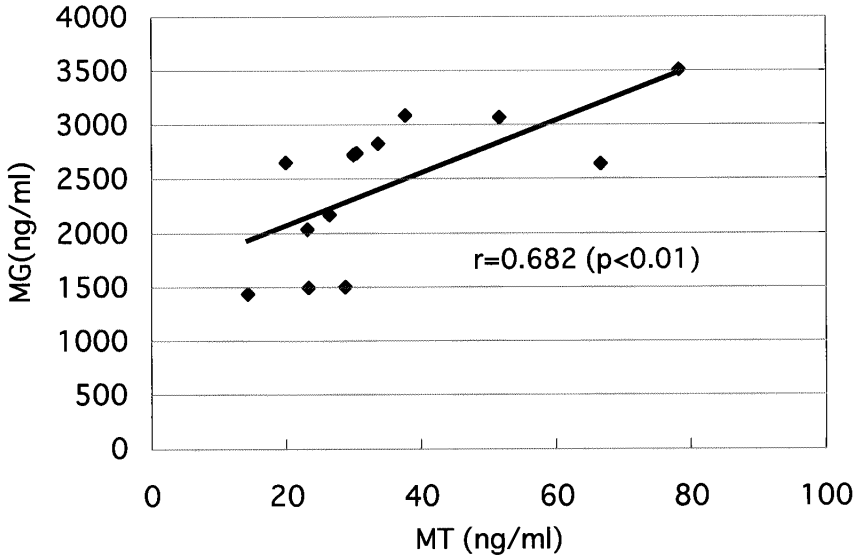

Fig. 1. Relationship between MT and MG in human amniotic fluid.
Amniotic fluids (n=12) without blood contaminants were collected during Caesarian section.

Table 1. Urinary MG, zinc, and MT levels

Urine	β 2-MG (ng/mg creatinine)	Zn (μg/mg creatinine)	MT (ng/mg creatinine)
Control	53.6 ± 6.1	0.5 ± 0.04	17.4 ± 1.5
Early	96.3 ± 9.9	1.2 ± 0.1	40.7 ± 4.4 *
Middle	156.2 ± 36.1	1.2 ± 0.1	31.3 ± 8.0
Late	444.9 ± 91.0	0.9 ± 0.1	22.5 ± 4.1
Newborn infant	9364.1 ± 1651.1 **	16.8 ± 2.6 **	161.2 ± 40.1 *

*p<0.05, **p<0.01 vs. control group. mean ± S.E.

Table 1 shows urinary levels of MT, zinc, and MG. The amount of MT in urine increased significantly in the Early stage of pregnancy. High levels of MT were also observed in the urine of newborn infants. The amount of MG increased dependent on the stage of pregnancy, and very high levels were observed in the urine of newborns. Zinc levels were shown to peak at the Middle period of pregnancy, and high content of zinc was also detected in the urine of newborn infants. A positive relation between MT and zinc was found in the urine of newborn infants ($r=0.574$, $p<0.05$), but not in pregnant and control women. In addition, there was no relation between MT and MG in newborn infants, pregnant women or control women.

Fig. 2 shows serum zinc levels in both pregnant women and newborn infants. Levels decreased in the Late stage of pregnancy in comparison with values for healthy young women, and after parturition, decreased still more.

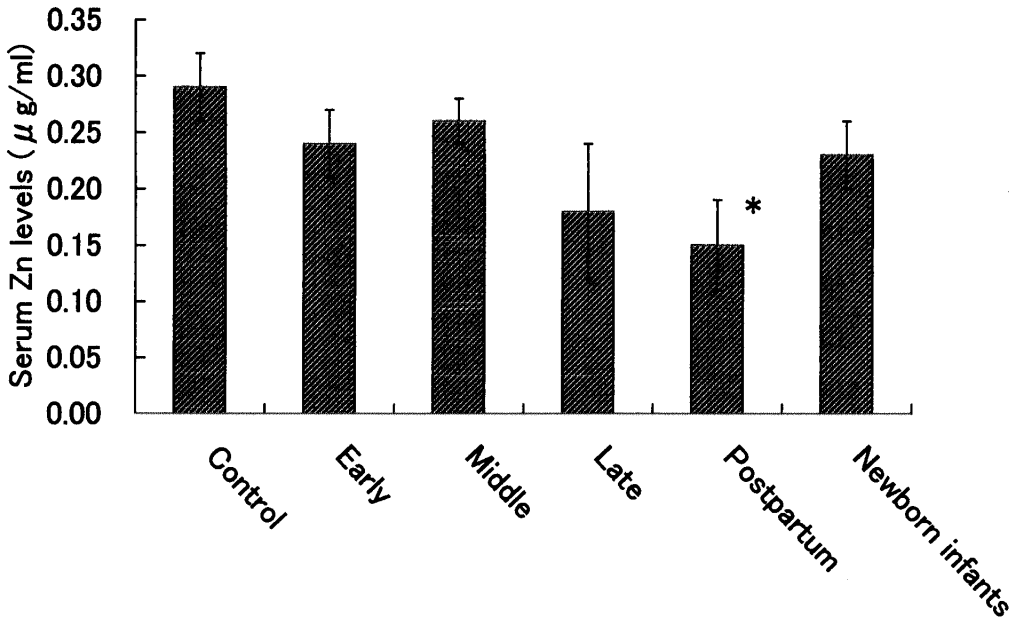

Fig. 2. Zinc concentration in serum of pregnant and postpartum women and infants. Control group: female volunteers, n=14, 18-22 years old, Early group: n=22, 5-21 weeks, Middle group: n=31, 22-28 weeks, Late group: n=24, 28-40 weeks, postpartum group: n=20, 2 days after parturition, newborn infants: n=12, 0-6 days. *$p<0.05$ vs. control group.

DISCUSSION

Homeostatic conditions for zinc in serum are required during pregnancy, especially during the last trimester and postpartum as shown in Fig. 2, as zinc deficiency reduces not only the growth of the placenta and maternal tissues but also fetal growth and development [4,15]. However, dietary zinc intake is affected by alcohol, tobacco, drugs, and so on[4]. In addition, iron supplementation also reduces the plasma zinc level[16], although iron is normally used to improve the symptoms of hypochromic anemia, which occurs sometimes during pregnancy.

MT acts in the absorption, secretion and storage of zinc in zinc homeostasis [6-8]. Carey et al.[11,17] observed that enhanced MT levels reduced zinc transfer from dam to fetus and increased fetal abnormalities using ethanol-administered MT-null and normal mice. There is some possibility that zinc deficiency occurs in the fetus when production of MT is induced in maternal tissues rashly. However, a large amount of MT is produced in various fetal organs, and as shown in Fig. 2, urinary MT had a good relationship with zinc in infant urine. MT may act in the absorption, secretion and storage of zinc in the organs of the fetus and infant.

In human placenta, MT was identified in fetal amniotic cells, syncytial trophoblasts and villous interstitial cells, and in maternal decidual cells[13]. The MT in placenta is suggested to restrict cadmium and to increase zinc and copper transport from the mother to the fetal body[13,18,19]. In addition, it is speculated that the increase of MT in human amniotic fluid

depends on the level of cadmium[20,21]. Amniotic fluid consists of fetal urine, when the gestation is over 20 weeks. The increase in MT in amniotic fluid was well correlated with the level of MG, but not the zinc concentration in the present study. The increase of MT in amniotic fluid may reflect not only metal metabolism but also renal function of the fetus, although we did not measure cadmium levels in amniotic fluid. In addition, the increase of MT level in maternal urine was not followed by the increase of pregnant periods. Excess production of MT may be controlled in maternal organs.

In conclusion, it is necessary to observe the zinc concentration in serum during pregnancy, especially the last trimester and postpartum, as the level of zinc changes with the progression of pregnancy and as zinc may be required by the fetus and infant. In addition, MT may be important in the fetus and infant for maintenance of the zinc concentration. And MT may be produced in the fetal kidney to protect the kidney.

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