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Yuji Hitamatsu* Katsuto Eguchi†

Kaoru Sekiba‡

*Okayama University,

†Okayama University,

‡Okayama University,

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Abstract

Polyamines have a close relationship with rapid cell proliferation. We measured polyamine levels in amniotic fluid, maternal plasma and urine during normal pregnancy. Plasma putrescine, spermidine and spermine gradually increased in the third trimester and reached the highest concentration at the end of pregnancy. There was a significant correlation between the level of these polyamines and the level of plasma estradiol and progesterone. In urine, putrescine and spermine increased with the progress of gestation and reached the highest level during the 8th to 10th months of gestation. In amniotic fluid, putrescine and spermidine concentrations were significantly high in the first trimester and decreased in the other trimesters, whereas spermine showed no significant change. Polyamine concentrations in maternal plasma and urine appear to reflect not only fetal metabolic changes but also the metabolic changes of the pregnant women, and to be influenced by several hormones which increase during pregnancy. Polyamines in amniotic fluid mainly reflect activated fetal metabolism and may be useful as biochemical indicators of fetal growth.

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ALTERATIONS IN POLYAMINE LEVELS IN AMNIOTIC FLUID, PLASMA AND URINE DURING NORMAL PREGNANCY

Yuji HIRAMATSU, Katsuto EGUCHI and Kaoru SEKIBA

Department of Obstetrics and Gynecology, Okayama University Medical School, Okayama 700, Japan

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Abstract. Polyamines have a close relationship with rapid cell proliferation. We measured polyamine levels in amniotic fluid, maternal plasma and urine during normal pregnancy. Plasma putrescine, spermidine and spermine gradually increased in the third trimester and reached the highest concentration at the end of pregnancy. There was a significant correlation between the level of these polyamines and the level of plasma estradiol and progesterone. In urine, putrescine and spermine increased with the progress of gestation and reached the highest level during the 8th to 10th months of gestation. In amniotic fluid, putrescine and spermidine concentrations were significantly high in the first trimester and decreased in the other trimesters, whereas spermine showed no significant change. Polyamine concentrations in maternal plasma and urine appear to reflect not only fetal metabolic changes but also the metabolic changes of the pregnant women, and to be influenced by several hormones which increase during pregnancy. Polyamines in amniotic fluid mainly reflect activated fetal metabolism and may be useful as biochemical indicators of fetal growth.

Key words : polyamine, pregnancy, plasma, urine, amniotic fluid.

Polyamines are polycationic substances which are widely distributed in biological materials. In animal tissues spermidine, spermine and their precursor putrescine are the three main polyamines (1). It is well established that cellular polyamine levels and ornithine decarboxylase, the rate limiting enzyme of polyamine biosynthesis, increase dramatically during cell growth and differentiation (2-4).

There are a number of reports concerning the role of polyamines in nucleic acid synthesis and cell proliferation (2-4). Clinically, there has been considerable interest in investigating polyamines as possible markers of malignancy (3), following an initial study which showed an increased amount of polyamines in urine of cancer patients (5). However, there have been few reports of polyamines concerning fetal growth, even though active cell proliferation occurs during pregnancy (6-8).

The present study was undertaken to determine polyamine levels in amniotic fluid, plasma and urine of normal pregnant women, and to evaluate the possiblity of polyamines playing a role in fetal growth.

MATERIALS AND METHODS

Collection of amniotic fluid, plasma and urine. All samples are obtained from normal pregnant

women without complications, such as toxemia of pregnancy, diabetes mellitus or other hormonal diseases.

Amniotic fluid samples in the first trimester were obtained from pregnant women who was operated because of uterine myoma. The samples in the second trimester were obtained by ultrasonography-guided transabdominal amniocentasis from patients who required chromosomal examination. The samples in the third trimester were obtained by amniotomy at delivery.

Plasma samples of pregnant women were collected from the cubital vein with a heparinized syringe at 10-12 a.m. Amniotic fluid and plasma were stored at -40℃ immediately after the collection until the analysis.

The 24 h urine specimens were collected in plastic bottles containing 10 ml of concentrated HCl and analyzed in the same day.

Preparations of amniotic fluid, plasma and urine samples. One ml of amniotic fluid and plasma was treated with an equal volume of cold 10 % trichloroacetic acid (TCA) containing 5×10^{-5}M internal standard. Triethylene tetramine, a polyamine synthesized by Dr. Samejima, Josai University, was used as the internal standard.

The samples were placed in an ice bath for 30 min in order to precipitate protein, and then centrifuged for 10 min at room temperature. The supernatant was decanted, and the pellet was resuspended in 0.5 ml of 10 % TCA and recentrifuged. The supernatant was added to previous wash and hydrolyzed in 6N HCl for 24 h at 110℃. After hydrolysis, the sample was evaporated using a centrifugal evaporator (Model RD-21, Yamato Scientific Co., Tokyo). The residue was reconstituted in 100 μl of 0.1N HCl, and 20 μl of aliquots were loaded on the analyzer. The recovery rates of putrecine, spermidine and spermine were more than 95 % by this procedure.

One ml of collected urine was mixed with an equal volume of 10 % TCA containing 8×10^{-5}M internal standard and hydrolyzed in 6N HCl for 24 h at 110℃. Twenty μl aliquot of the samples were loaded on the analyzer. At the same time, urinary creatinine was measured using the Creatinine Test Wako (Wako Pure Chemicals Industries, Osaka).

The concentrations of polyamines in amniotic fluid and plasma were expressed as pmol/ml, and urinary polyamines were expressed as mg/g creatinine.

Analysis of polyamines. For the present analysis, we used the following apparatuses: an HLC-805 amino acid analyzer, an HLC-805 reactor (Toyo Soda Co., Tokyo), an FS-970LC fluorometer (Kratos Inc.) and a recorder. Separation of each polyamine was achieved on a TSK-IEX-215SC column (Toyo Soda Co.) with a stepwise elution of two buffers, followed by fluorometric detection with O-phtalaldehyde (OPA). The detailed composition of buffers, OPA reagent and analytical conditions were reported previously (8).

In this system, all polyamines and the internal standard were separated well and rapidly (Fig. 1). Elution times were 30, 25 and 30 min for plasma, urine and amniotic fluid, respectively. Polyamines in all samples were determined with a coefficient of variation under 4 %.

Assay of estradiol, progesterone and human chorionic gonadotropin (HCG). Plasma estradiol and progesterone were measured in 25 randomly selected women whose polyamine concentrations had already been measured. Plasma estradiol and progesterone were assayed using an Estradiol I-125 Kit and a Progesterone I-125 Kit (Commissariat a l'Energie Atomique, France), respectively.

The urinary HCG titer was measured in 40 pregnant women after 6 to 15 weeks of gestation in order to investigate the correlation between polyamines and HCG. The samples were suitable for study because a marked change in urinary HCG titer occurs during this period. Urinary HCG titer was measured using a Mochida Pregnancy Test "Gonavislide" (Mochida Pharmaceutical Co., Tokyo).

For statistical analysis, Student's t-test was used.

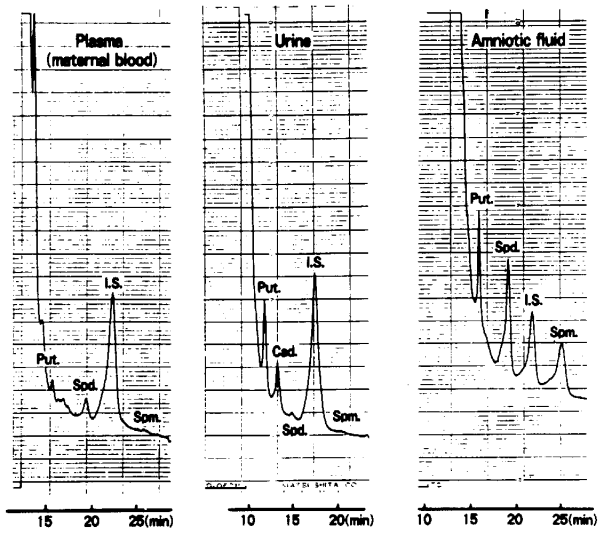

Fig. 1. Chromatograms of polyamines in plasma, urine and amniotic fluid. Put. =putrescine, Cad. =cadaverine, Spd. =spermidine, I.S. =internal standard, Spm. =spermine.

RESULTS

Alterations in plasma polyamine levels during normal pregnancy. Changes in plasma polyamine levels during normal pregnancy are shown in Fig. 2. Putrescine, spermidine and spermine did not show any remarkable changes throughout the first and second trimesters. Each polyamine level, however, gradually increased in the third trimester and reached the highest concentrations at the end of pregnancy. Each polyamine level at the end of pregnancy was significantly higher than those

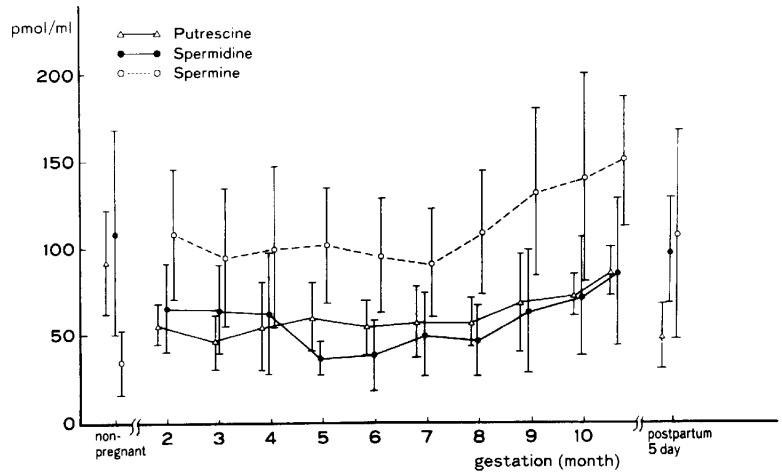

Fig. 2. Alterations in plasma polyamine levels during normal pregnancy. There were 218 samples analyzed. Each point shows the mean ± S.D. of 8-28 patients. Each value at the end of pregnancy (putrescine 85.08 ± 26.97, spermidine 85.18 ± 45.08, spermine 149.64 ± 38.50 pmol/ml) was significantly higher than in the first and second trimesters.

in the first and second trimesters ($p<0.02$, $p<0.01$ or $p<0.001$). Especially, spermine showed a marked change and had the highest levels of all the polyamines throughout pregnancy. Its value in the third trimester was approximately 1.5-fold higher than in the first and second trimesters.

Correlations between plasma polyamines, estradiol and progesterone. It is well known that a number of hormones stimulate polyamine biosynthesis in various target organs (3). Since estrogen and progesterone show marked changes during pregnancy, the correlations between polyamines and these hormones were analyzed.

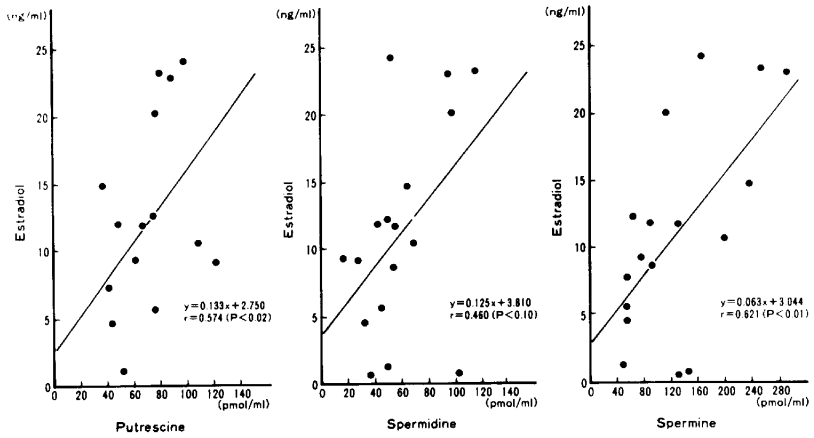

Fig. 3. Correlations between plasma polyamines and estradiol. Plasma estradiol showed a significant correlation with putrescine ($p<0.02$) and spermine ($p<0.01$).

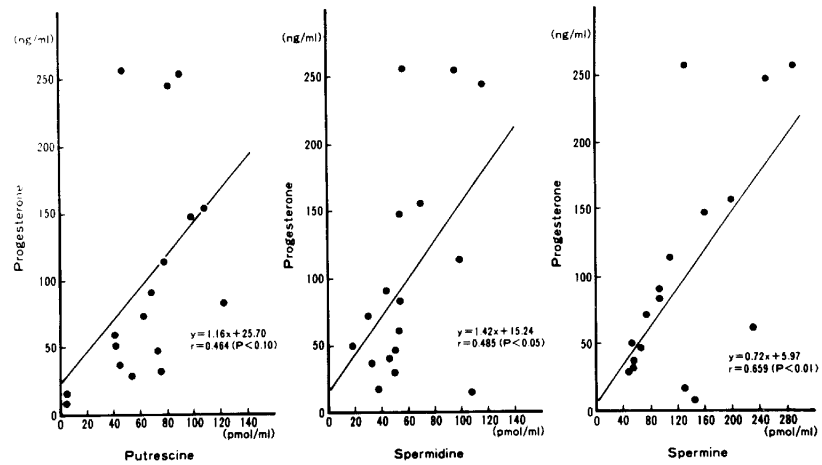

Fig. 4. Correlations between plasma polyamines and progesterone. Plasma progesterone showed a significant correlation with spermidine ($p<0.05$) and spermine ($p<0.01$).

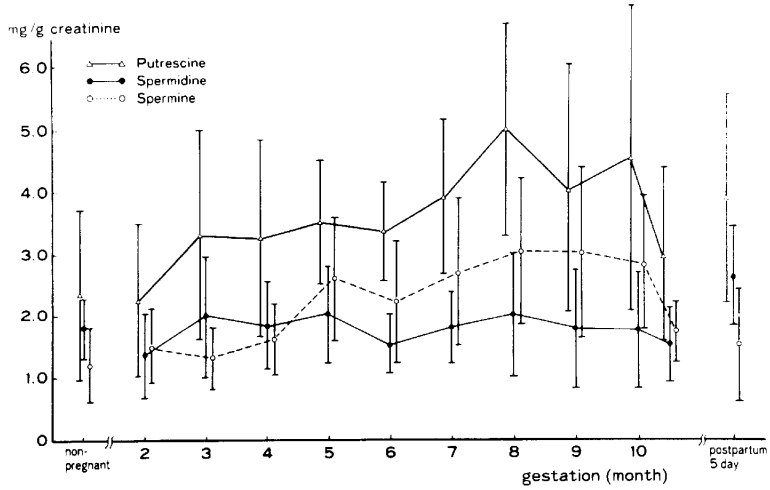

Fig. 5. Alterations in urinary polyamine levels during normal pregnancy. There were 256 samples analyzed. Each point shows the mean ± S.D. of 10-36 patients.

Plasma estradiol showed a significant correlation with putrescine ($p<0.02$) and spermine ($p<0.01$) (Fig. 3). Plasma progesterone showed a significant correlation with spermidine ($p<0.05$) and spermine ($p<0.01$) (Fig. 4).

Alterations in urinary polyamine levels during normal pregnancy. Changes in urinary polyamine levels during pregnancy are shown in Fig. 5. Putrescine and spermine levels were higher in pregnant women than in non-pregnant women and gradually increased with the progress of gestation. From the 8th to 10th month of gestation, putrescine and spermine showed the highest levels, and putrescine levels significantly differed from those non-pregnant women ($p<0.001$ or $p<0.01$). However, there were no statistically significant changes in spermidine and spermine throughout pregnancy. At the end of pregnancy, all polyamine concentrations decreased.

In urine, putrescine characteristically had the highest levels in any stages of pregnancy, and the change in its level was the most significant.

Correlations between urinary polyamines and urinary HCG titers. The correlations between urinary polyamines and urinary HCG titers were studied in 40 pregnant women during 6 to 15 weeks of gestation. There were no significant correlations between the polyamines and HCG titers (data not shown).

Concentrations of polyamines in amniotic fluid. Polyamine concentrations in amniotic fluid are shown in Fig. 6. The putrescine concentration in the first trimester was 2-2.5 times higher than in other trimesters ($p<0.05$ or $p<0.001$). There also was a significant difference between the second and the third trimester ($p<0.001$). The spermidine concentration was found to be significantly ($p<0.02$ or $p<0.01$) elevated at the first trimester. There were no significant differences in the spermine concentration.

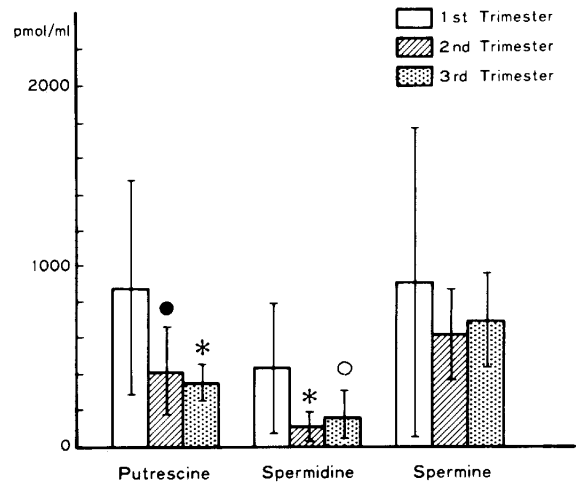

Fig. 6. Concentrations of polyamines in amniotic fluid. Values are the mean ± S.D. (pmol/ml). Seven samples were taken in the 1st trimester, 10 in the 2nd trimester and 35 in the 3rd trimester. Significantly different from the 1st trimester: ● $p<0.05$, ○ $p<0.02$, ＊ $p<0.01$.

DISCUSSION

The precise physiological function of polyamines is still uncertain, but it has been well established that polyamines have an essential role in cell growth, and that their accumulation proceed to the synthesis of nucleic acid and protein. A striking but transient increase in ornithine decarboxylase prior to the elevation of polyamines (2-4).

A systemic study concerned with the concentration of polyamines as a parameter of cell proliferation was carried out in developing chick embryos by Raina (9). He reported that changes in polyamine concentrations correlated with those of protein and RNA. In human liver, the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase and levels of putrescine are also increased during the proliferating stage of fetal development (10).

In this study, changes in extracellular polyamine levels, which appear to reflect changes in cell kinetics, were investigated. There are, however, two problems in discussing changes in extracellular polyamine levels during pregnancy. One is that we must consider the feto-placental-maternal unit. The fetal metabolic products first appear in the umbilical blood and excreted by the maternal kidney. Furthermore, maternal blood and urine reflect not only the fetal metabolic changes but also the metabolic changes of the pregnant women. The other problem is the influence of hormones which show marked changes during pregnancy. In addition, it is well known that a number of hormones, including growth hormone, cortisol, ACTH, lutenizing hormone, estradiol, HCG and insulin, stimulate polyamine biosynthesis in various target organs (3).

The changes in polyamine levels in amniotic fluid found in the present study generally concur with the earlier findings of Chan *et al.* (11). Namely, the level of each polyamine in amniotic fluid was highest during the first trimester. This phenomenon appears to reflect the marked mitotic activity and proliferation of cells during fertilization, implantation and organogenesis. In the second and third trimesters, each polyamine concentration was lower than in the first trimester. This change might be influenced by polyamine oxidase which increases with the progress of pregnancy (12).

In maternal plasma, each polyamine concentration remained at most the same level from the beginning of pregnancy to the end of the second trimester, then increased until partrition, and decreased during the puerperium. This pattern seems to reflect the rapid fetal growth in this period of gestation but it may also reflect changes in the levels of estrogen and progesterone during pregnancy, since a significant correlation was noted between polyamine levels and levels of these hormones (Fig. 3, 4). Thus a number of hormones, including estradiol and progesterone, may influence polyamine metabolism during pregnancy.

The concentration of putrescine is characteristically high in urine, and putrescine and spermidine levels were slightly elevated in the latter half of the pregnancy. There were large individual deviations in urinary polyamine levels, and the alteration with pregnancy was not as marked as in plasma and amniotic fluid.

Russell *et al.* (6) reported that each urinary polyamine has a very high transient peak around 12 weeks of gestation. However, we did not find the same phenomenon. Since the alteration of polyamine levels that they reported corresponded well with the pattern of urinary HCG, we investigated the correlation between urinary polyamines and urinary HCG titers, but failed to find a constant correlation. While the reason for this discrepancy is obscure, we suppose that the dramatic changes in polyamines with fetal growth are not easily reflected in maternal urine, because maternal urine is the final product influenced by fetal metabolic changes.

Recently, high urinary polyamine concentrations in preterm infants (13), and low serum and urinary polyamine concentrations in children with growth hormone deficiency, Turner's syndrome and short stature (14), have been reported. Furthermore, we found that fetal blood polyamines show dramatic changes during pregnancy (15).

In summary, the present results suggest that polyamines play important roles in human growth, although the precise functions are still unknown. Polyamines in amniotic fluid appear to reflect fetal growth better than those in maternal plasma and urine. Studies are ongoing to determine the polyamine concentrations in various abnormal pregnancies, and to explore the possibility of polyamines being used as markers of fetal growth.

REFERENCES

1. Bachrach, U.: *Functions of Naturally Occurring Polyamines.* Academic Press, New York and London,

pp. 1-20, 1973.
2. Raina, A., Eloranta, T., Pajura, R.L., Mäntyjärvi, R. and Tuomi, K.: Polyamines in rapidly growing animal tissues. In *Polyamines in Biomedical Research*, ed. J.M. Gaugas, John Willey & Sons, Chichester, pp. 35-49, 1980.
3. Jänne, J., Pösö, H. and Raina, A.: Polyamines in rapid growth and cancer. *Biochim. Biophys. Acta* **473**, 241-293, 1978.
4. Snyder, S.H., Kreuz, D.S., Medina, V.J. and Russell, D.H.: Polyamine synthesis and turnover in rapidly growing tissues. *Ann. N.Y. Acad. Sci.* **171**, 749-771, 1970.
5. Russell, D.H.: Increase polyamine concentrations in the urine of human cancer patients. *Nature* **233**, 144-145, 1971.
6. Russell, D.H., Giles, H.R., Christian, C.D. and Campbell, L.J.: Polyamines in amniotic fluid, plasma, and urine during normal pregnancy. *Am. J. Obstet. Gynecol.* **132**, 649-652, 1978.
7. Casti, A., Reali, N., Orladini, G., Ferrari, B., Salvadori, B. and Bernasconi, S.: Blood polyamines in human development. In *Advances in Polyamine Res.* ed. C.M. Caladarera, V. Zappia and U. Bachrach, Ravin Press, New York, pp. 463-472, 1981.
8. Hiramatsu, Y., Eguchi, K., Yonezawa, M., Hayase, R. and Sekiba, K.: Alterations of red blood cell's polyamines during pregnancy and neonatal period. *Biol. Neonate* **40**, 136-144, 1981.
9. Raina, A.: Studies on the determination of spermidine and spermine and their metabolism in the developing chick embryo. *Acta Phisiol. Scand.* **60** (suppl 218), 1-81, 1963.
10. Sturman, J.A. and Gaull, G.E.: Polyamine biosynthesis in human fetal liver and brain. *Pediatr. Res.* **8**, 321-327, 1974.
11. Chan, W.Y., Seale, T.W., Shulka, J.B. and Rennert, O.M.: Polyamine conjugates and total polyamine concentrations in human amniotic fluid. *Clin. Chim. Acta* **91**, 233-241, 1979.
12. Illei, G. and Morgan, D.M.L.: Polyamine oxidase activity in amniotic fluid and fetal membrane. *Br. J. Obstet. Gynecol.* **87**, 413-415, 1980.
13. Perman, M., Chan, W.Y., Ramadan, T.Z. and Rennert, O.M.: Urinary polyamines in preterm infants. *Clin. Chim. Acta* **113**, 1-6, 1981.
14. Rudman, D., Kutner, M.H., Chaula, R.K., Goldsmith, M.A., Blackston, R.D. and Bain, R.: Serum and urine polyamihes in normal and in short children. *J. Clin. Invest.* **64**, 1161-1168, 1979.
15. Hiramatsu, Y., Eguchi, K. and Sekiba, K.: Polyamine concentrations in the umbilical blood. *Acta Med. Okayama.* **39**, 265-273, 1985.