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Serum Levels of Hormone Profiles and Urinary Excretion of Calcium in Premenopausal and Postmenopausal Women in Taiwan

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Abstract : *Serum concentrations of FSH, LH, and estradiol, and urinary excretion of calcium were assessed in 20 premenopausal women and 48 postmenopausal women. FSH, LH and estradiol were measured by immunoradiometric assays (IRMA). Urinary calcium (Ca) levels were measured by atomic absorption method, and creatinine (Cr) by Jeffe reaction. The mean concentrations of FSH and LH in postmenopausal women were 79.4 mIU/ml and 23.9 mIU/ml, which were significantly higher than 4.6 and 5.7 mIU/ml in premenopausal women, respectively. On the other hand, the mean concentration of estradiol in postmenopausal women was 11 pg/ml, which was much lower than of 122 pg/ml in premenopausal women. The mean Ca/Cr molar ratio in postmenopausal women was 0.232, whcih was significantly higher than that of 0.143 in premenopausal women. In conclusion, lower serum concentration of circulating estradiol is one of the possible factors for the increased excretion of calcium and osteoporosis in postmenopausal women. (Thai J Obstet Gynaecol 1991;3:1-5.)*

Key words : serum concentration, FSH, LH, estradiol, urinary calcium, premenopause, postmenopause

Menopause is characterized by a marked decrease in the serum level of estrogens. In menopause, estradiol production drops to 10 to 20%, and estrone to about 20 to 30% of the level of reproductive women, respectively[1,2]. Osteoporosis occurs very often in Caucasian postmenopausal women[3]. Bone loss in postmenopausal women starts at about the time

of menopause and proceeds at a rate of about 1% per year[4]. Moreover, involution of trabecular bone starts as early as 30-35 years of age, and will increase in the early menopausal period to 6%, to reach a maximum in bilaterally oophorectomized women with 9%[5]. Decrease or cessation of ovarian estrogen production seems to be associated with the increased rate of bone loss in postmenopausal women[6].

In the present study, we will measure the serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and estradiol, and urinary excretion of calcium in premenopausal and postmenopausal women. We will analyse the relationship of serum levels of hormone profiles and the enhanced bone loss in postmenopausal women.

Materials and Methods

This study comprised of two groups of women, premenopausal and postmenopausal. There were 20 patients in the premenopausal group. They had regular menstruation cycles and had no endocrine or major medical diseases. The range of age was 25 to 40 years with a mean age of 32 years. The total number of postmenopausal women was 48, including 28 with surgically castrated menopause and 20 with natural menopause. The age range was 31 to 64 years with a mean age of 49 years.

Venous blood and fasting morning urine were collected at day 7 to 10 of follicular phase in premenopausal women. In postmenopausal women the specimens were collected at least one year after natural menopause or two months after surgical castration. No hormone agents were taken at least 2 months before collection of specimens.

Serum concentrations of FSH, LH, and estradiol were measured by immunoradiometric assays (IRMA) with FSH MAIAclone kit (Code 13101, Serono Diagnostics), LH MAIAclone kit (Code 13201, Serono Diagnostics) and Biodata Estradiol MAIA kit (Code 12264, Serono Diagnostics), respectively. Urinary calcium concentration was measured with atomic absorption. Urinary creatinine concentration was measured with Jeffe reaction. The calcium/creatinine concentration ratio was expressed in molar ratio.

The data obtained was analyzed by Student's t-test. A p-value less than 0.05 was considered statistically significant.

Results

The serum levels of FSH, LH, and estradiol are shown in Figure 1. The mean concentrations of FSH and LH in postmenopausal women were 79.4 mIU/ml and 23.9 mIU/ml, which are significantly higher than 4.6 and 5.7 mIU/ml, respectively, in premenopausal women. On the other hand, the serum concentration of estradiol in postmenopausal women was 11 pg/ml, which is much lower than that of 122 pg/ml in premenopausal women.

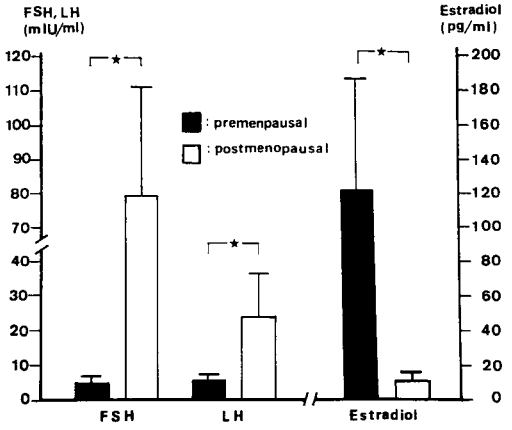

Fig. 1 Comparison of serum concentrations of FSH, LH and estradiol between premenopausal and postmenopausal women. Each column and vertical bar represent mean and one standard deviation.
* = $p<0.01$.

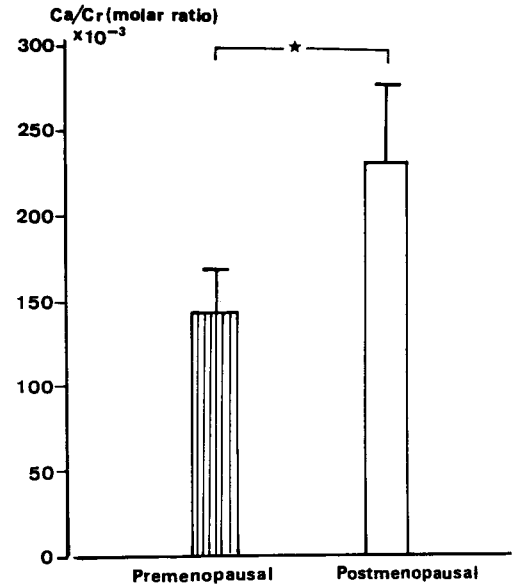

Fig. 2 Comparison of urinary calcium/creatinine molar ratio between premenopausal and postmenopausal women. Each column and vertical bar represent mean and one standard deviation.
* = $p<0.01$.

Figure 2 shows the fasting urinary calcium/creatinine (Ca/Cr) molar ratio in these two groups of women. The mean Ca/Cr molar ratio in postmenopausal women was 0.232, which is significantly higher than that of 0.143 in premenopausal women.

Discussion

From the present study, we found that the rate of urinary excretion of calcium was much higher in postmenopausal women. The Ca/Cr ratio was 0.232 in postmenopausal women, which is almost two-fold higher than that of 0.143 in premenopausal women. On the other hand, the circulating level of estradiol in postmenopausal women was less than 12% of the premenopausal level. The Ca/Cr molar ratio seems to be inversely related with the circulating concentration of estradiol. The pituitary gonadotropins (FSH, LH) levels in postmenopausal women were much higher than those in premenopausal women. The FSH/LH ratio was greater than 3 in postmenopausal women, which is quite contrary to that in the premenopausal group.

Nordin et al[7,8] pointed out that the rise in fasting urine calcium reflected an increase in total bone resorption. In 1977, Lindsay et al[9] also reported that the fasting urinary Ca/Cr ratio was inversely related to the circulating estradiol concentration. A lower amount of circulating estrogen was considered as one of the factors responsible for the increased urinary

calcium excretion and the negative calcium balance[10]. Young and Nordin[11] suggested that loss of estrogenic activity at natural or artificial menopause resulted in increased bone resorption. Throughout life the bones exchange calcium with the extracellular volume, i.e. from the bones as a result of resorption and to the bones as a result of bone formation. Osteoclasts are responsible for bone resorption and osteoblasts for bone formation. Bone resorption is always followed by a period of bone formation[12]. The pathogenesis of the postmenopausal bone loss is still not fully understood, although its close relationship to female sex hormones is widely established. Recent studies have demonstrated evidence for the presence of estrogen receptors in normal human osteoblast-like cells, and have pointed out that estrogen can act directly on human bone cells by a classical estrogen receptormediated mechanism[13,14].

In conclusion, the present study suggests that a lower serum concentration of circulating estradiol is one of the possible pathophysiologic factors for the increased urinary excretion of calcium and osteoporosis in postmenopausal women.

Acknowledgement

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Investigation of Human Fetal Thymus Blood Supply

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Abstract : *The fetus in utero is in a sterile environment, protected from contact with most microorganisms. The thymus plays a crucial role in the maturation of T-lymphocytes, as the generation of immunocompetent T-cells requires an intrathymic differentiation of precursor cells. Thymic pathological processes are sometimes healed only by its extirpation, so knowledge of thymus vascularisation is of great importance. The aim of this study was to investigate anatomic variations of human fetal and newborn's thymus vascularisation. The research comprised 20 newborns who died due to intracranial hemorrhage, 3-20 days after birth. Contrast (gelatin ink) injected into the abdominal aorta filled thymic arteries retrogradely. Lateral branches of the inferior thyroid artery were found to provide arterial blood to the thymus cervical part in 11 cases, while in the other nine, branches of internal thoracic arteries were found. After investigating the thoracic part of the thymus gland we found that the internal thoracic artery supplied ipsilateral lobes in 16 newborn, while in one case, the left one was found to have branches for both lobes. The thoracic part of the thymus had arterial vascularisation from pericardial vessels in two cases while in one the presence of an odd interlobal artery originating from brachiocephalic trunk was observed. Knowledge of anatomic variations of thymus arterial vascularisation is of great importance in invasive diagnostic procedures as well as in surgical intervention. (Thai J Obstet Gynaecol 1991;3: 7-11.)*

Key words: human thymus, arterial vascularisation, anatomy

At about the sixth week of gestation, the thymus is generated from the epithelium of the third and fourth pharyngeal pouches[1]. The thymus gland is the only lymphatic organ in which reticular cells are of endodermal origin. It is sited retrosternally, in the superior mediastinum. Lymphatic tissue in the thymus gland is grouped in two lateral lobes, with connective or glandular tissue between them. It is most active during fetal development and in early postnatal life. The thymus increases in size rapidly in utero, more gradually until puberty and then involutes during adult life. Previously de-

scribed as an endocrine organ, the thymus is now stated to play an important role in T-lymphocyte maturation processes. Contemporary investigations regard the thymus as part of the hypothalamus-pituitary-thymus-gonadal axis and as a participant in the regulation and modulation of neuroendocrine functions of human beings throughout their whole life[2]. Besides being phy - siological, the thymus gland can also undergo accidental involution (due to some pregnancy-associated pathological processes, malnutrition, infection or intoxication). It is also suggested that the thymus can play a certain role in the pathogenesis of autoimmune diseases. All the thymic functions and the pathological conditions mentioned are in close relation to its vascularisation, innervation and lymphatic drainage. The thymic vascular compartment is recognized for uptaking monoclonal antibodies[3]. Important variations of thymus arterial blood supply exist, caused by its mobility during development and various positions in the superior mediastinum. This includes various arterial origins of thymic arteries, their ipsi- and bilateral lobar supply as well as intrathymic vascular pattern[4]. The aim of this study was to investigate anatomic variations of thymic arterial vascularisation that are of great importance for invasive diagnostic procedures as well as for thoracic surgery.

Materials and Methods

The investigation was performed in the Clinic of Gynaecology and Obstetrics, Belgrade University Clinical Center, on 20 fetuses or newborns (11 males and 9 females). The newborns had died due to intracranial hemorrhage 3-20 days after birth. The gelatin-ink was injected through the abdominal aorta retrogradely, with subsequent filling of thymic arteries (Figure 1). The sternal and costal cartilages were carefully dissected away, preserving the internal thoracic arteries and their thymic branches. The thymus gland was freed of the capsule and its arterial vascular pattern was examined.

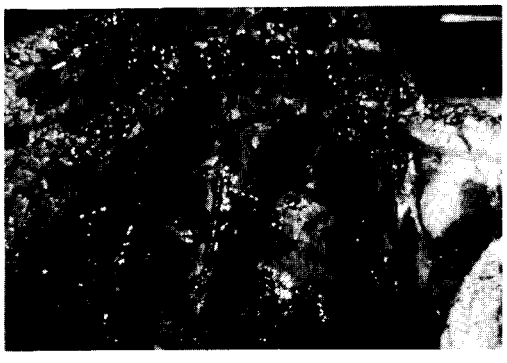

Fig. 1 Fetal specimen with injected internal thoracic overlying the thymus gland.

Results

Topographically the thymus is divided by the suprasternal notch into the cervical and thoracic parts. Investigating the origin of the arterial thymic vessels for the cervical part we found it to be the inferior thyroid artery in

11 cases and the internal thoracic artery in 9 cases (Figure 2), with ipsilateral arterial supply from both origins. The thoracic part of the thymus received branches mainly from the internal thoracic artery (17 cases) (Figure 3), the pericardial vessels (3 cases) and from the brachiocephalic trunk (1 case). The bilateral arterial blood supply was stated in only two cases, one coming from the left internal thoracic artery, and the other from the odd interlobal artery of brachiocephalic trunk origin.

Fig. 2 Thymic artery emerging from the internal thoracic artery and entering the superior pole of thymic lobe.

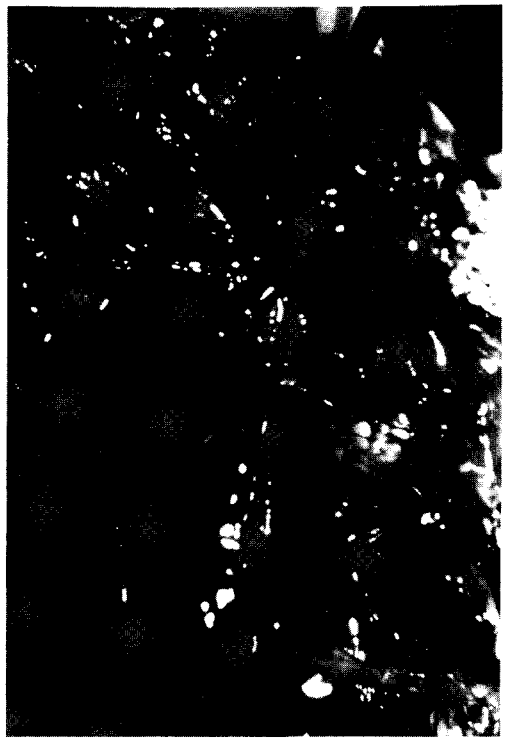

Fig. 3 Odd interlobal artery giving few branches for thymic lobe.

Discussion

The study of thymic arteries vascular pattern gives reliable data of its functional capacity and physiological status. Thymic vascularisation undergoes consistent changes through development and in certain pathological conditions, such as pneumonia[5]. The vascular pattern in the involuted thymus becomes irregular and the vessels are tortuous[6]. Because of that, investigations of human fetal thymus give more precise information on their arterial blood supply in comparison to the adult thymus.

The thymic arterial circulation

has been proved to be very vulnerable and sensitive to exsanguination. It can be reduced up to 52% in status of hemorrhagic shock[7].

Thymic arteries mainly originate from the internal thoracic artery, less frequently from the inferior and rarely from the superior thyroid artery. Exceptional cases with subclavian, carotid or brachiocephalic origin have been described[1].

One among the earliest descriptions of the thymus blood supply was given by Testut[8]. In his profound studies, the atypical origin of the odd thymic artery from the brachiocephalic trunk was noted supplying both lobes, which we have also found.

Yamasaki[9] studied the thymic vessels in adult cadavers and fetuses. He described the disappearance of thymic arteries in earlier stages of deveolpment, being replaced by the superior thyroid artery. He named this branch, A. thymica suprema. No such case was found in our material. The same author found that the arterial systems of the thyroid and the thymus gland are largely dependent on the existence of the abnormalities of the constant arteries and of the anomalous arteries. These anatomical variations showed higher frequencies in fetuses than in adults. In his subsequent study, he found that the middle thymothyroid artery showed the highest frequency, compared to the superior and middle thymic artery. The supreme thymic and the thyroid ima artery arising from the internal thoracic artery were found to be extremely rare[10].

In our previous investigation we found that the thymus cervical part was supplied by the inferior thyroid artery almost three times more frequently compared to the internal thoracic artery[11]. Our reinvestigations showed a nearly equal participation of those two origins in providing arterial blood to the cervical thymus.

Kato[6] described thymic arteries in mice which branched into arterioles as they entered the thymic parenchyma. In our human material, also, no extrathymic branching was found.

In conclusion, one can say that significant variations of human fetal thymus arterial system exist and that its knowledge is necessary for thymus pathophysiological processes investigations, diagnostic procedures, as well as for surgical intervention.

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The Effects of Thymus Peptides and Oxytocin on Motility and Vitality of In Vitro Cultivated Human Spermatozoa

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Abstract : *The thymus is, according to the contemporary opinion, a part of the hypothalamus-pituitary-thymus-gonadal axis and participates in the regulation of the endocrine reproductive functions in human organisms. Besides specific hormone synthesis, neurohormone substances are made in the subcapsular zone (oxytocin, vasopressin). In human seminal plasma of fertile males, high levels of thymic hormone thymosin α 1 have been found, which influence the maturation processes and germinal cells function. High concentrations of neurohormone oxytocin (OT) have been recorded in the male reproductive tract. The results of the investigations confirm the influence of human fetal thymus extract (FTH) and juvenile calf thymus extract (JCTH) on the motility and vitality of human sperm. These extracts, which beside other biologically active substances, contain thymosin α 1 and oxytocin, were added to human sperm as a medium addition for sperm washing (SWM); after that, the investigation of their number and some vitality parameters was done: motility (M), progressive motility (PM), velocity motility (VM) and velocity of the progressive motility (VPM). Preliminary results of these studies indicate that the thymic factors application can be of use in the investigation and clinical treatment of male infertility, as well as in in vitro fertilization (IVF) procedures. (Thai J Obstet Gynaecol 1991; 3:13-19.)*

Key words : human spermatozoa, motility, sperm washing medium (SWM), fetal thymus extract (FTH), juvenile calf thymus extract (JCTH), oxytocin

The thymus is an important organ of the immunologic and endocrinologic system of the human organism. It has been proved that it plays an important role in the reproductive function of female and male gonads. A series of peptide substances are produced in the thymus and hormone bioactivity is the subject of intensive investigations. The most important among them are: thymosin α 1, thymosin β 4 and thymulin [1,2]. In the

subcapsular zone of the human and animal thymus neurohormones oxytocin and vasopressin have been found in relatively high concentrations [3-5]. Hall and Mcgillis[6] noted in their investigations the thymus-pituitary-gonadal axis existence. Neonatal thymectomy of experimental animals leads to gonadal dysgenesis[7]. Findings of the significantly decreased thymosin ß 4 concentrations in postmenopausal females and in those with surgically removed ovaries are very interesting[8]. Of special importance are the investigations which confirmed the presence of significantly higher thymosin concentrations in seminal plasma of fertile males in relation to the group of infertile ones, that is the confirmation of thymosin α 1 and fertility correlation[9]. Knowing that the thymus can be of the utmost importance in clinical andrology and reproductive endocrinology, we started an investigation of the influence of the thymus peptides from the extracts of human and juvenile calf thymus, and of the oxytocin on spermatozoa in *in vitro* conditions.

Materials and Methods

In cooperation with the Clinic of Gynaecology and Obstetrics, University Clinical Center in Belgrade, Institute of Biology and Human Genetics in Belgrade and Institute for Immunology and Thymus Reseach in Bad Harzburg, Federal Republic of Germany, we studied the influence of bioactive peptides (and neuropeptides) of human fetal and juvenile calf thymus extracts, and of synthetic oxytocin on 50 semen samples in *in vitro* conditions. Semen analysis was made after ejaculation of liquefaction[10]. Sperm washing media containing a series of substances such as enzymes, amino acids, antibiotics, hormones, serum, etc., were used in the investigation. Spermatozoal washing was performed twice and then centrifugation on 300 g. All testings were done in an incubator with controlled temperature, humidity and gaseous content, under the same conditions[11]. Hemocytometry was used for separation of motile and nonmotile spermatozoa. Such a "swim up" procedure enables separation of highly motile spermatozoa from the ones with less satisfactory vital abilities although it has been found that this procedure is definitely harmful for a certain number of spermatozoa, and their number was decreased after the washing[12]. The following parameters were determined in washed semen: number of vital spermatozoa, motility (M%), progressive motility (PM%), velocity motility (VM μm/s) and velocity progressive motility (VPM μm/s).

For our investigtions we used a) standard medium, b) standard medium (10 ml) and juvenile calf peptide thymus extract (1.5 mg), c) standard medium (10 ml) and lyophylization preparation of human fetal thymus extract (1.5 mg), and d) standard medium (10 ml) and oxytocin (Syntocinon) 1 IU.

These parameters were determined in washed sperm after 1hr,

24hr, and 48hr and the samples labelled with (a.) represented the control group to the standard medium for cultivated sperm.

The obtained information was numerically expressed and analyzed by X^2 and Student t-test on an IBM computer.

Results

Spermatozoa counting was determined in 50 analyzed ejaculations of 50 patients after the use of standard medium for spermatozoa washing (cultivation). After an hour, their number was 35 ± 15 x 10^6 ml, while higher values were found in native preparation (before sperm washing, 45 ± 10 x 10^6 ml, Table 1, Figure 1). Spermatozoa count was somewhat lower in samples cultivated in media added with juvenile calf (JCTH) or human fetal thymus extract (FTH) or synthetic oxytocin (30 ± 9 x 10^6 ml, Table 1, Figure 1). The increase of spermatozoal motility was observed in the medium with oxytocin (M=56± 11%) as well as human fetal thymus extract (M=55 ± 11%) (Table 1, Figure 2). Progressive motility (PM) was of higher values with oxytocin application (40 ± 12%) than with FTH (32 ± 15%, Table 1, Figure 3). Velocity motility (VM) was the highest in samples cultivated with oxytocin (39 ± 6 μm/s) and FTH (39 ± 11 μm/s, Table 1, Figure 4). Statistical analysis indicates borderline significance of these values. Special attention was paid to the investigation of progressive velocity motility (VPM). We found no significant differences between VPM of spermatozoa cultivated in standard medium and in native sperm (47 ± 12 μm/s, 40 ± 8 μm/s, respectively), while the oxytocin initiated a significant increase of this parameter (49± 5 μm/s, $p<0.05$, Table 1, Figure 5).

The values of the investigated parameters decreased 24 hours after the cultivation. However, it is interesting to point out that the motility was significantly higher in samples with JCTH (20 ± 6% and FTH (17 ± 5%), than in those with standard medium (9 ± 7%) and oxytocin (5 ± 2%) ($p<0.05$, Table 1, Figure 2). Progressive motility (PM) had almost the same values in all of the media, except in FTH application, where it was somewhat increased (10 ± 4%, Table 1, Figure 3). Spermatozoa cultivation in the media with JCTH and FTH caused increased velocity motility (VM) in comparison to oxytocin and standard medium (Table 1, Figure 4). Velocity of the progressive motility (VPM) was higher in samples with JCTH and FTH (22 ± 10 μm/s and 25 ± 9 μm/s, respectively), than in those with standard medium and oxytocin (18 ± 12 μm/s and 15 ± 8 μm/s, Table 1, Figure 5).

The fact that the greatest spermatozoa count was recorded 48 hours after the cultivation in samples with JCTH (7 ± 1 x 10^6 ml), with significance of $p<0.05$, is of particular interest (Table 1). Motility was the highest in preparations with FTH and JCTH (7 ± 2% and 10 ± 4%), especially in

Table 1 Influence of medium ingredients on the main spermatozoa features

		ST	JCTH	FTH	OT
Number	0h	45 ± 10	45 ± 10	45 ± 10	45 ± 10
$\bar{X}$ ± SD	1h	35 ± 15	27 ± 16	30 ± 9	30 ± 9
(x 10^6 / ml)	24h	12 ± 6	8 ± 2	9 ± 3	2 ± 1
	48h	5 ± 2	7 ± 1	2 ± 2	2 ± 2
M	0h	46 ± 5	46 ± 5	46 ± 5	46 ± 5
$\bar{X}$ ± SD	1h	52 ± 12	50 ± 7	55 ± 11	56 ± 9
(%)	24h	9 ± 7	20 ± 6	17 ± 5	5 ± 2
	48h	5 ± 1	10 ± 4	7 ± 2	2 ± 1
PM	0h	27 ± 8	27 ± 8	27 ± 8	27 ± 8
$\bar{X}$ ± SD	1h	30 ± 15	29 ± 13	32 ± 15	40 ± 12
(%)	24h	6 ± 5	9 ± 3	10 ± 4	5 ± 1
	48h	7 ± 2	8 ± 2	9 ± 2	4 ± 1
UM	0h	31 ± 7	31 ± 7	31 ± 7	31 ± 7
$\bar{X}$ ± SD	1h	36 ± 10	35 ± 10	39 ± 11	39 ± 6
(μM/s)	24h	11 ± 4	15 ± 6	20 ± 7	11 ± 5
	48h	11 ± 5	20 ± 7	19 ± 2	15 ± 7
UPM	0h	40 ± 8	40 ± 8	40 ± 8	40 ± 8
$\bar{X}$ + SD	1h	47 ± 12	45 ± 9	35 ± 7	49 ± 5
(μM/s)	24h	18 ± 12	22 ± 10	25 ± 9	15 ± 8
	48h	18 ± 9	25 ± 6	11 ± 6	15 ± 1

ST - Standard medium
FTH - Fetal thymus extract
Number - Number of spermatozoa
PM - Spermatozoa progressive motility
VPM - Spermatozoa velocity progressive motility
Oh - Before adding ingredients
1h - One hour after adding ingredients
JCTH - Juvenile calf thymus extract
OT - Oxytocin
M - Spermatozoa motility
VM - Spermatozoa velocity motility
24h - hours after adding ingredients
48h - hours after adding ingredients

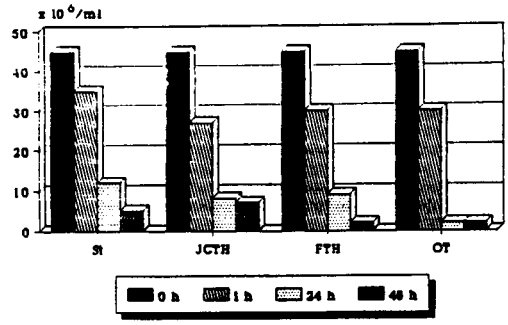

Fig. 1 Number of spermatozoa.

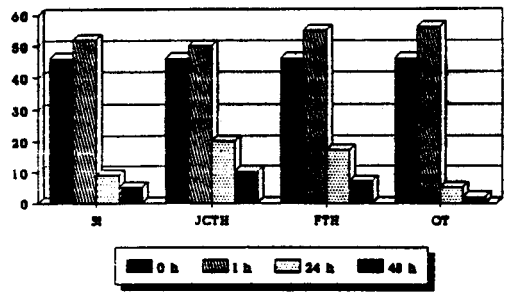

Fig. 2 Spermatozoa motility.

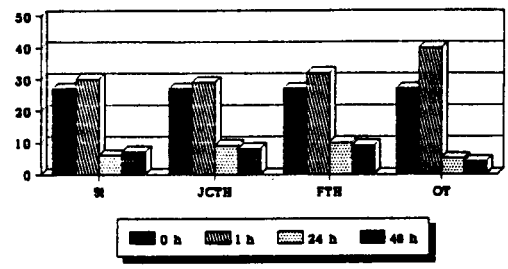

Fig. 3 Spermatozoa progressive motility.

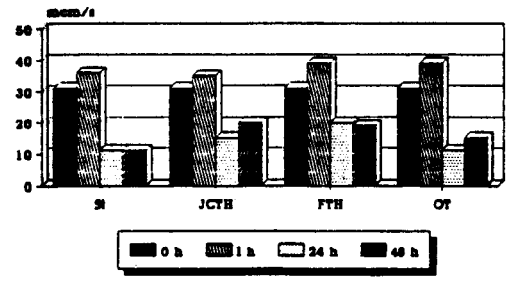

Fig. 4 Spermatozoa velocity motility.

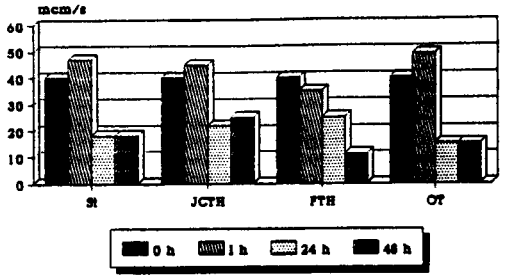

Fig. 5 Spermatozoa velocity progressive motility.

relation to the values in oxytocin media (2 ± 1%), (Table 1). PM and VM were of higher values with JCTH and FTH presence. VPM was statistically significantly higher in preparations treated with JCTH (25 ± 6 μm/s), than in other media ($p<0.05$) (Table 1, Figures 4, 5).

Discussion

Parameters of functioning or to be more precise of spermatozoal vitality investigated in this study are of crucial importance for *in vitro* and *in vivo* fertilization. Today, when we have knowledge of numerous physiological properties and constituents of seminal fluid in the procedures of spermatozoal preparation for intrauterine insemination (IUI), in vitro fertilization (IVF), gamete intra-Fallopian tubes transfer (GIFT), media containing different bioactive substances (amino acids, enzymes, antibiotics, nutritive factors, hormones, etc.) are used for "swim up" [13, 14, 15]. Recent studies proved high thymosin α 1 levels in seminal plasma, in correlation to the spermatozoal count, viability and

motility in fertile males, in comparison to infertile ones, having more prominently decreased parameters[9].

After standard medium usage for semen washing, spermatozoal count was of much lower value after 24hr and 48hr since only more vital ones remained (managed to survive). Global motility and velocity of the progressive motility were increased after an hour in relation to the values prior to incubation.

After spermatozoal cultivation in medium with juvenile calf thymus extract (JCTH) was added, a longer period of global motility maintenance was observed, especially of VPM, in respect to the spermatozoa in standard medium.

Addition of human fetal thymus extract stimulates global motility at first, then the progressive one, and also the spermatozoal velocity motility, in all of the testing time intervals. JCTH and FTH represent total thymus extracts, which, besides the specific thymus peptides, contain neuropeptides, such as oxytocin. Therefore, synthetic oxytocin addition in standard medium for spermatozoal washing (cultivation) causes characteristic changes. Significant increase of M, PM and VPM of the spermatozoa values occurred an hour after addition of a medium with oxytocin, while after 24hr and 48hr sudden decrease of spermatozoal vitality developed. Oxytocin stimulates intercelluar glucose oxidation initiating a prompt energetic potential release significant for cellular development and activity[17].

Investigations of Maggi et al[16] indicate that oxytocin receptors, which play a very complex role in human reproductive endocrinology, are present in the male genital tract.

Media for spermatozoal cultivation, which contain specific thymus peptides and neuropeptides, manifest influence on their motility and vitality in *in vitro* conditions, and is of great importance for future investigations in reproductive clinical endocrinology.

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The 26th British Congress of Obstetrics & Gynaecolgy will take place at the University of Manchester Institute of Science and Technology, Manchester, England from 7-10 July 1992. Information available from BCOG Secretariat, 65, West Drive, Cheam, Sutton, Surrey, SM2 7NB, UK

The Expression of Corticotropin-Releasing Hormone Gene and its Immunohistochemical Analysis in Human Trophoblast of Normal Pregnancy and Trophoblastic Disease

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Abstract : *Immunoreactive corticotropin-releasing hormone (IR-CRH) in maternal plasma increases progressively with pregnancy and rapidly declines after delivery. So the production site of IR-CRH is thought to be placenta. We studied the expression of CRH gene and its immunohistochemical localization in human developing chorionic tissue, amniotic membranes, uterine myometrium and fresh surgical specimen of hydatidiform mole with the methods of Northern blotting and avidin-biotin complex staining. The results are 1) the CRH mRNA were identified in third trimester and term placentae, but could not be demonstrated in first and second trimesters placentae, 2) the CRH mRNA expresses in amniotic membranes but not in myometrium of normal term pregnancy, 3) IR-CRH localization was demonstrated in the cytotrophoblast of placenta and decidua of first trimester, and in the amniotic membranes of term pregnancy and, 4) the CRH mRNA and its immunohistochemical localization was not detected in trophoblast of hydatidiform mole. These results suggest that the sources of increased IR-CRH in human plasma and amniotic fluid during pregnancy are placenta, decidua and amniotic membranes, and that the gene expression and secretion / storage ratio of placental CRH increases as pregnancy advances. The expression in trophoblast of hydatidiform mole may be suppressed because of the characteristic change of trophoblast. (Thai J Obstet Gynaecol 1991;3: 21-30.)*

Key words: corticotropin-releasing hormone (CRH), northern blotting, immunohistochemical localization

It was formerly supposed that corticotropin-releasing hormone (CRH) exists in the hypothalamus[1,2]. In 1981, ovine-CRH was first purified from ovine hypothalami by Vale et al[3] and its amino acid sequence as well as the primary structure of the biosynthetic precursor of human CRH (hCRH) was

determined[4]. Subsequently, Sasaki et al[5] reported that the concentration of immunoreactive (IR)-CRH in human plasma progressively increased during pregnancy and rapidly declined after delivery, and the production site of the IR-CRH is supposed to be placenta. To date, the expression of hCRH gene in term placenta has been reported[6], but there has been no systemic study at various stages of pregnancy, nor in amniotic membranes, uterine myometrium or trophoblastic disease. In this paper, we studied hCRH in these tissues at both gene and protein levels by the use of molecular biological and immunohistochemical methods.

Materials and Methods

Tissues

Placentae of 6, 10, 14, 15, 17 and 23 weeks of pregnant women were obtained by therapeutic abortion, and that of 28 weeks of pregnancy was obtained from a woman who went into premature delivery. Placentae, amniotic membranes and uterine myometrium of 40 weeks gestation were obtained from women undergoing repeat, or elective cesarean section. Hydatidiform mole, from a patient who was diagnosed at 10 weeks of pregnancy, was sampled as the specimen of trophoblastic disease. All patients gave their informed consent to participate in this study. Hypothalamic and liver explants were obtained from male Sprague Dawley rats (180-200g weight, Japan Animal Farm).

Tissues for hybridization experiments were frozen in liquid nitrogen and stored -80°C until RNA isolation. For immunohistochemical stain, samples of 6 and 40 weeks of pregnancy, and of hydatidiform mole were fixed with 10% formalin /PBS for 48 hours.

Northern blotting

CRH probe

The inserted DNA segment containing the gene for the CRH precursor, which was subcloned into plasmid of pBR 322, was 3.8kb genomic DNA[4]. The 265hp DNA fragment which contains a part of first intron[5] and, part of second exon was separated from Pvull digestion of the plasmid, and used for CRH probe.

Labelling of probe

The probe was labelled with (α-^{32}p) dCTP (3000Ci/mmol/MEN) by multipriming system kit (Boehringer Mannheim). The specific activity of the radiolabelled probe was 0.8-2.0x10^9 cpm/μg DNA.

RNA isolation

About 1.0g tissues were homogenized with 9ml of 4M guanidine isothiocyanate (GIT) in Polytron on ice, then the guanidine lysed samples were laid on the top of 5ml of 5.7mol cesium chrolide buffer and spun for 20 hours at 15°C, 27000rpm. The ob-

tained total RNA samples were quantified by UV absorption at 260nm.

Northern blot hybridization

5.0μg of either total RNA were denatured in resin treated glyoxal mixture at 65°C for 1 hour, and electrophoresed on 1% agarose gel in 10mmol sodium phosphate buffer (pH 7.0), then transferred to Hybond nitrocellulose filter (Amersham, Japan). Prehybridization was performed in 0.5ml/cm^2 of a solution containing 2xSSC (1xSSC = 0.15M sodium chloride, 0.015M sodium citrate), 50mmol Tris (pH 7.4), 1 x Modified Denhardt, 1mol NaCl, 10mmol EDTA, 0.1% SDS and 10μg/ml denatured salmon sperm DNA. Prehybridization was done at 50°C for 1 hour. Hybridization was done in the same buffer (0.1ml/cm^2) plus 10^{6}cpm/ml of radioactive probe and incubated at 65°C for 24 hours. The filters were then washed in 4xSSC, 0.1%SDS for 10 minutes at room temperature followed by 2xSSC, 0.1%SDS at 50°C for 10 minutes, and 0.1xSSC, 0.1%SDS at 61°C for 30 minutes. Filters were then blotted dry and exposed for 24 hours using intensifying screen (Fuji Film Co.,Japan).

Immunohistochemistry

Formalin-fixed tissues were dehydrated and embedded in paraffin. Sections were cut at 4μm, deparaffinized and rehydrated. Immunostaining, using anti-hCRH antibody as first antiserum, was carried out by the avidin-biotin complex method[7], using Vectastin ABC kits (Vector Laboratories Inc., Burlingame, CA). CRH antiserum was raised in rabbits as described in detail elsewhere[8]. Diamino-benzidin tetrahydrochloride was used as peroxidase substrate. The sections were treated with 0.3%H_2O_2 in methanol and 3% normal rabbit serum to reduce nonspecific background staining and block endogeneous peroxidase activity. In addition to the immune serum diluted 1:100, normal rabbit serum was used for controls. In order to allow a reliable histological study of the tissues, hematoxilin-eosin stain was also carried out in each tissue.

Results

Northern blotting

Fig. 1 shows the northern blot analysis of rat hypothalamic and human 40 weeks placental RNA. A single band was hybridized with the probe in rat hypothalamic RNA lane (lane 4) as positive control and in the placental RNA lane (lane 3);both sizes were about 1300 nucleotides, as previously described[6]. No hybridized material was detected in molar RNA lane (lane 1), 10 weeks placental RNA lane (lane 2) and rat liver RNA lane (lane 5) as negative control. Northern blot hybridization analysis of RNA from developing placentae of various stages is shown in Fig. 2; pregnancy 6 weeks (lane 1), 10 weeks (lane 2), 14 weeks (lane 3), 15 weeks (lane 4), 17

weeks (lane 5), 23 weeks (lane 6), 28 weeks (lane 7) and 40 weeks (lane 8). No positive band was detected except in 28 and 40 weeks placental RNA lane. The analysis of placental, amniotic membraneous and uterine myometrial RNA of term pregnancy is also shown in Fig. 3. Lane 1 shows placenta, lanes 2 and 3 show amniotic membranes and uterine myometruim. Single positive bands about 1.3kb were detected in amniotic membraneous and placental RNA lane of 40 weeks of pregnancy, but not in uterine myometrial lane.

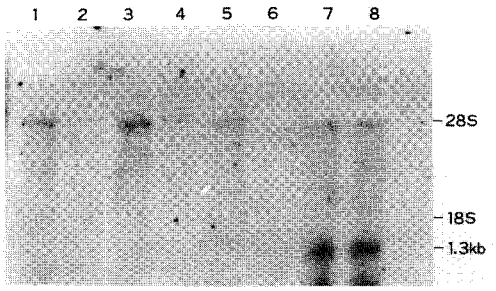

Fig. 2 Northern blot hybridization analysis of RNA from developing placentae. Lane 1, human placenta of 6w pregnancy; 2, 10w; 3, 14w; 4, 15w; 5, 17w; 6, 23w; 7, 28w; 8, 40w. As size markers, 28S and 18S ribosome RNA were shown in right side.

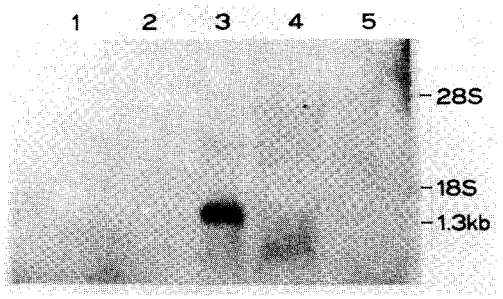

Fig. 1 Northern blot hybridization analysis of rat hypothalamic, liver, human placental and molar RNA. Lane 1, human hydatidiform mole; 2, human placenta (10w); 3, human placenta (40w); 4, rat hypothalamus (positive control); 5, rat liver (negative control). 28S and 18S ribosome RNA were used as size markers, and internal standards (right side).

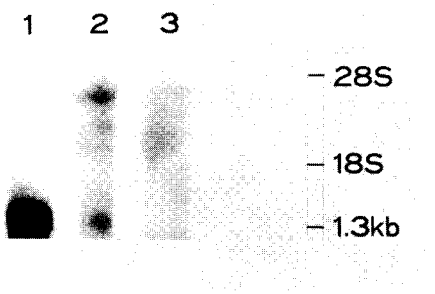

Fig. 3 Northern blot hybridization analysis of placenta, amniotic membranes and uterine myometrium. In right side, 28S and 18S rRNA were shown as size markers.

Immunohistochemistry

In the placenta of 6 weeks of pregnancy, the cytotrophoblast layer beneath the syncytiotrophoblast was evenly stained as shown in Fig. 4. The glandular epithelium was stained in the decidua, and the stromal cells were diffusely positive as well (Fig.5). In term placenta, the staining reaction was negative (Fig.6), but the amniotic membranes were heavily stained (Fig.7). In the tissue of hydatidiform mole, no evidence of immunostain was seen (Fig.8).

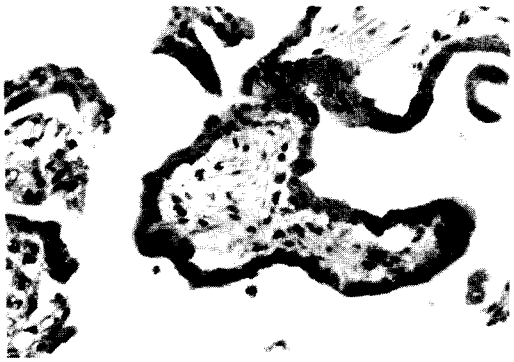

Fig. 4 Immunohistochemical analysis of normal placenta, six weeks of gestation.
(A) Haematoxylin-eosin staining,
(B) control staining,
(C) staining with anti-CRH immune serum. In (C), note that positive reaction is confined to the cytotrophoblast. Original magnification ; x200.

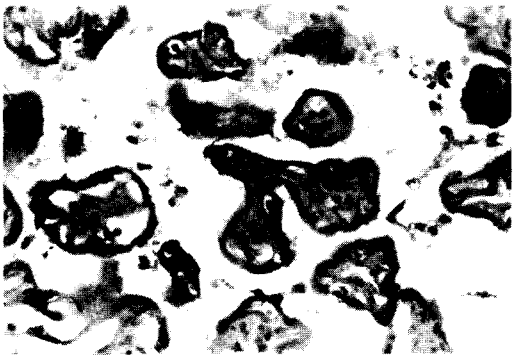

Fig. 5 Normal decidua, six weeks of gestation. Staining is as in Figure 4. Note the positive reaction both in the glandular epithelium and in many stromal cells. Original magnification ; x200.

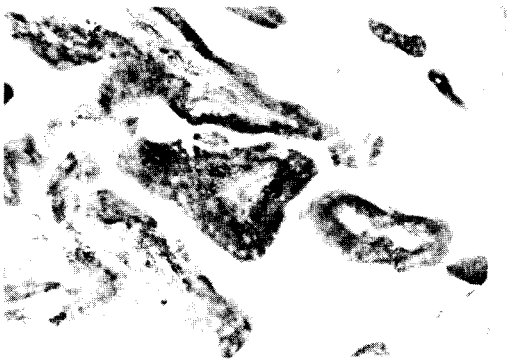

Fig. 4 (B)

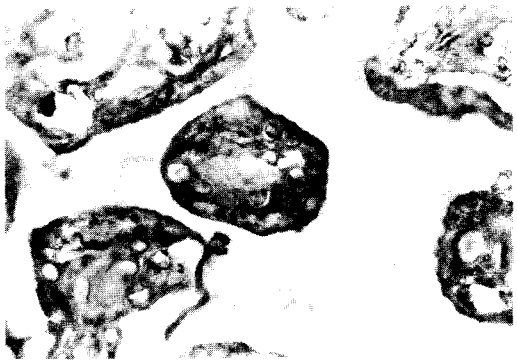

Fig. 5 (B)

Fig. 4 (C)

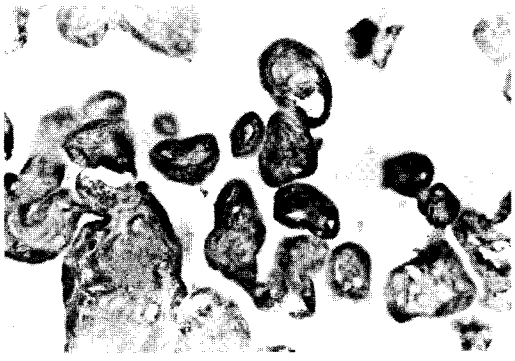

Fig. 5 (C)

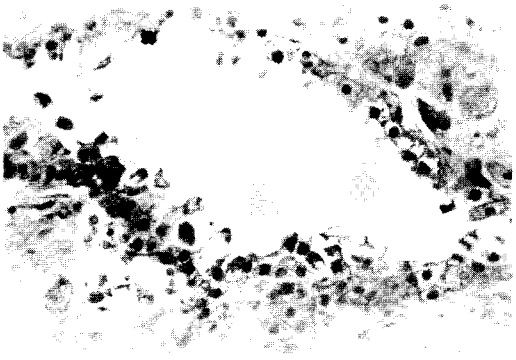

Fig. 6 Normal placenta, 40 weeks of gestation. Note that there is no staining reaction in frame C, where the immune serum has been used. Original magnification ; x200

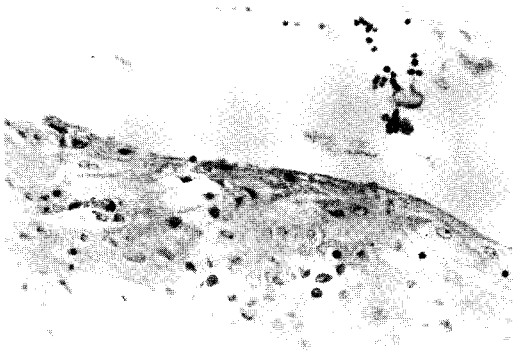

Fig. 7 Normal amniotic membranes, 40 weeks of gestation. Note the strong CRH reaction in the epithelium in frame C. Original magnification ; x200

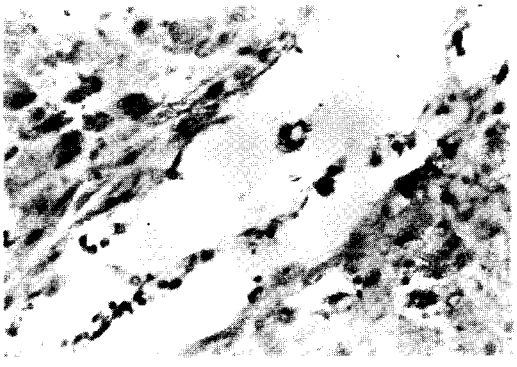

Fig. 6 (B)

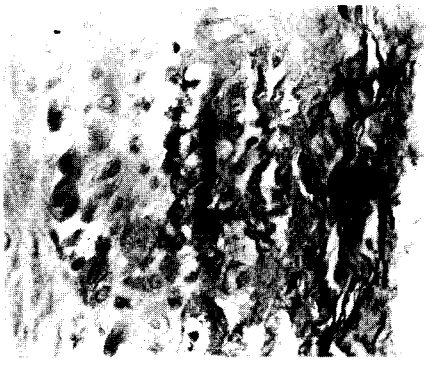

Fig. 7 (B)

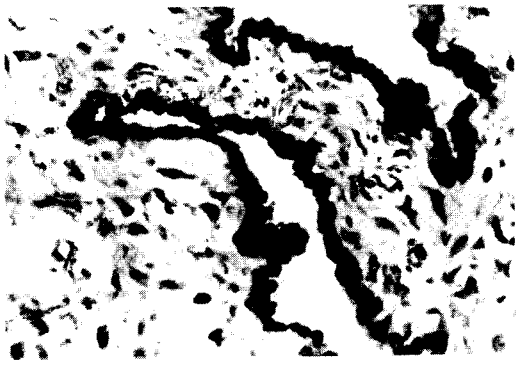

Fig. 6 (C)

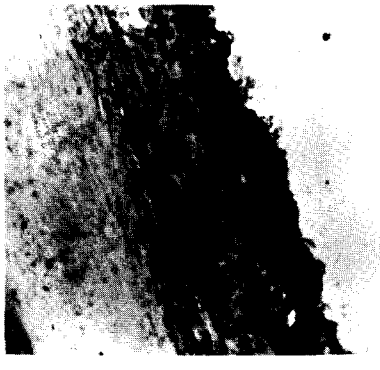

Fig. 7 (C)

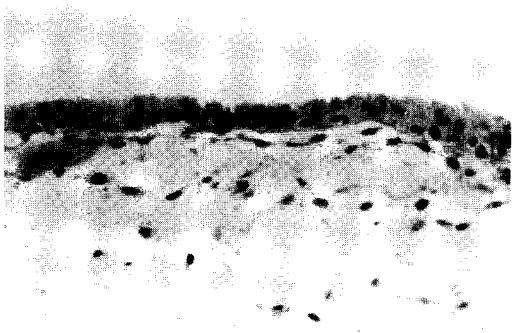

Fig. 8 Hydatidiform molar tissue, 10 weeks of gestation. Note that there is no staining reaction in frame C where anti-CRH serum has been used.

Fig. 8 (B)

Fig. 8 (C)

Discussion

Although term placenta has a high concentration of IR-CRH (2-25μg/placenta)[9], there has been no systemic study for the expression of hCRH in placenta at various stages of pregnancy, nor in amniotic membranes, uterine myometrium or trophoblastic disease. In this report, we show that the expressions of hCRH gene in placenta do not appear clearly at 6, 10, 14, 15, 17 and 23 weeks of pregnancy, but become evident after 28 weeks of gestation. Thus, it is supposed that the expression of CRH mRNA increases coincidental with the growth and maturation of the placenta. At term pregnancy, the expression of CRH gene in amniotic membranes, which is weaker than that in placenta, is also identified, but not in myometrium which is attached closely to the placenta.

IR-CRH localization was also demonstrated in the cytotrophoblast of placenta and decidua of early pregnancy, and in the amniotic membranes of term pregnancy. The observations that IR-CRH is localized in cytotrophoblast, but that CRH mRNA is below the limit of detection in first and second trimester placentae, support the notion that the localization of IR-CRH may be due to the storage of mitogen, not continuous gene expression. Similar phenomenon has also been observed on fibroblast growth factor in rat ovary[10]. It may be speculated that CRH is stored rather than secreted during early pregnancy, and the ex-

pression of CRH increases as pregnancy advances. At term pregnancy, the rate of CRH secretion exceeds storage and, thus, the proportion of stored CRH may be decreased in addition to the small proportion of cytotrophoblast. Consequently, the immunohistochemical finding of term placenta is negative, which is in accordance with previous reports[11,12].

On the basis of these results, it is sugested that the placenta and amniotic membranes of term pregnancy produce and secrete hCRH, and the production site of increased plasma CRH of pregnant women are these tissues. On the other hand, Laatikainen et al[13] reported that IR-CRH exists in amniotic fluid and it increases greatly during the latter half of pregnancy. It was shown that this increased IR-CRH in amniotic fluid is produced and secreted from amniotic membranes from our results together with their results.

A physiological role for the CRH during pregnancy has yet to be ascertained. It has been reported that CRH binding protein (CRH-BP) exists in human plasma and most of plasma CRH is bound to CRH-BP and inactivated[14,15]. Although a large amount of IR-CRH, most of which is produced from placenta, may be preferentially secreted into the maternal circulation, the influence of maternal pituitary-adrenal axis may be within the physiological range because a large proportion is bound to CRH-BP and inactivated. Another report suggested that CRH may be one of the initiators of labour, because the level of plasma IR-CRH of pregnant women who subsequently went into premature labour was raised several weeks before the onset of labour[16]. The increased IR-CRH and cortisol levels in amniotic fluid are accompanied with raised lecithin/sphingomyelin ratio and phosphatidylglycerol[13]. Similar observations have been reported on prolactin (PRL). IR-PRL in maternal plasma, as well as amniotic fluid increases during pregnancy, and human decidua contains high levels of IR-PRL[17]. It has also been suggested that PRL produced by the decidua is secreted through the fetal membranes into the amniotic cavity and accelerates fetal lung maturation. In view of these results, the CRH produced from amniotic membranes is probably secreted into the amniotic fluid and accelerates fetal maturation in obstetric stress by stimulating the fetal adrenal cortex to produce corticosteroids.

It is reported that the maternal plasma IR-CRH level is in normal non-pregnant range in hydatidiform mole[18]. However, there has been no molecular biological and immunohistochemical study of CRH in molar tissue. Our study shows that CRH mRNA and IR-CRH localization were not demonstrated in trophoblastic tissue of hydatidiform mole. Hydatidiform mole, which is a most common trophoblastic tumour, has a potential DNA synthesis and rapid growth and proliferation. In this tumour, CRH gene expression was probably suppressed due to the characteristic

change of trophoblast.

In conclusion, CRH is proved to be produced and stored in the placenta during normal pregnancy by both molecular biological and immunohistochemical techniques. Its production/storage ratio changes as pregnancy advances, smaller at early pregnancy and larger at term pregnancy.

Acknowledgement

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Gestational Choriocarcinoma with Brain Metastases : A Clinical Analysis

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Abstract : *This report reviews the clinical features of 14 choriocarcinoma patients with brain metastases treated from 1986 to 1990 at the University of Texas MD Anderson Cancer Center. The incidence is 5.3% of 264 gestational trophoblastic tumor patients. Brain metastases occurred after term delivery, abortion and hydatidiform mole in 9, 3, and 2 patients respectively. The group of term deliveries had a longer interval than the other two groups. Seven patients (50%) presented with neurological signs or symptoms, only one patient was asymptomatic. Five patients developed brain metastases during or after treatment with first line drugs. Abnormal serum : cerebrospinal fluid hCG ratio (less than 60 : 1) were found in only 3 patients (21%). Thirteen of 14 patients (93%) had accompanying pulmonary metastases. Brain metastases occurred equally in both cerebral hemispheres. The average modified WHO score was 15.2 (range 7-22). (Thai J Obstet Gynaecol 1991;3:31-37.)*

Key words : gestational choriocarcinoma, brain metastases

Gestational choriocarcinoma is a malignancy of the human placenta with the potential for rapid growth and widespread dissemination via hematogenous route, attributable to the erosive property of trophoblastic cells to invade adjacent tissues such as myometrium and blood vessels. Approximately one half of the cases were preceded by hydatidiform mole, with 25% following abortion, 22.5% following normal pregnancy, and 2.5% following ectopic pregnancy[1]. The incidence of gestational choriocarcinoma patients presenting with cerebral metastases not only varies between centers and geographic origins but also depends upon the surveillance and referral systems. These account for the incidence ranging between 7-28% with the higher ones in centers from the oriental countries[2-7]. The purpose of the current report is to analyze the clinical features of 14 patients with brain metastases of choriocarcinoma who were treated at the

University of Texas MD Anderson Cancer Center between February 1986 and July 1990.

Materials and Methods

A review was made of the medical records for all patients diagnosed as having choriocarcinoma with brain metastases to determine their age, parity, antecedent pregnancy, time interval from antecedent pregnancy to start of chemotherapy, pregnancy, presenting symptoms, initial urinary or serum hCG levels before treatment, prior chemotherapy, size, and sites of brain and other metastases. For assigning a score according to the World Health Organization (WHO) criteria[8], each chart was reviewed based on the prognostic factors except the information on ABO blood group which was not available in all cases. Hematologic profiles, chemical surveys, liver function test, urinalysis, and chest films were obtained in all patients at the time of initial examination. Radionuclide scan of the brain was used to identify cerebral metastases in most patients, but recently, computerized tomography (CT) and occasionally, magnetic resonance imaging (MRI) have been utilized for precise localization of the cerebral lesions. Other diagnostic radiologic tests were performed according to judgement of the attending physicians. Lumbar puncture with cerebrospinal fluid (CSF) examination for cytology, biochemistry and hCG measurement was usually employed unless there was evidence of increased intracranial pressure.

Results

The 14 patients of gestational choriocarcinoma with brain metastases constituted 5.3% of the total 264 patients with gestational trophoblastic tumor (GTT) and 20.5% of the total 68 patients with metastatic GTT treated in this institute during the study period. The characteristics and clinical profiles of the patients are listed in Table 1. The WHO score based on prognostic factors, excluding ABO blood group, is also assigned for each patient.

The ages ranged from 18 to 45 years (average = 30 years). The antecedent pregnancies included 9 term deliveries, 3 abortions, and 2 hydatidiform moles. The time between end of antecedent pregnancy and start of chemotherapy ranged from 6 months to 9 years (average = 30.8 months). The average interval was 35.5, 26.0 and 17.0 months in the groups of term deliveries, abortions and hydatidiform mole respectively. Seven patients presented with neurological sings or symptoms, most common were the signs of increased intracranial pressure such as severe headache, nausea, vomiting and impairment of consciousness or focal neurological signs caused by intracerebral lesions. Three patients presented with abnormal vaginal bleeding, all of them developed brain metastases while on treatment. One patient (KPC) was initially asymptomatic, but discovered lung nodule on

Table 1 Patient characteristics

Patients/ Year of admission	Age	History of pregnancy	Antecedent pregnancy	Interval[1]	Presenting symptoms	hCG[2] (mIU/ml)	Prior chemotherapy	WHO score[3]
LFT/1968	18	0010	Mole	10 mo	headache	+preg test	-	7
CTM/1972	37	6006	Term	9 mo	headache	-24 hr urine	-	11
KAD/1972	23	1001	Term	3 yr	lt. hemi-paresis	250	++	18
VAP/1972	23	0010	Mole	2 yr	chest pain	450,000*	++	16
MJH/1974	30	3003	Term	6 mo	Vg bleed & hemop[4]	850,000	++	20
JFR/1974	45	3003	Term	9 yr	Vg bleed & hemop[4]	180,000	-	21
GD/1975	31	5005	Term	5 yr	Vg bleed	150,000	++	22
PMG/1976	22	2002	Term	10 mo	seizure	14,000	-	15
TKT/1981	21	0010	Abortion	2 yr	headache	8,746*	-	12
RUN/1981	38	2002	Term	1 yr	lower GI bleed	739,200*	-	14
RDJ/1981	28	1021	Abortion	4 yr	Vg bleed	217	++	14
MLS/1982	38	6061	Term	6 yr	dizziness & diplopia	47,500	-	15
KPC/1988	22	1021	Abortion	6 mo	no symptom	1,048,800	-	12
SKS/1990	21	2002	Term	7 mo	coma	1,268,000	-	16

1 Time between end of antecedent pregnancy and start of chemogherapy
2 Initial serum hCG level before treatment; ++ = 2 or more drugs prior chemotherapy
3 World Health Organization scoring system based on prognostic factors
4 Vaginal bleeding & hemoptysis
* = Serum hCG: cerebrospinal fluid hCG ratio <60:1

routine preoperative chest film for cholecystectomy.

Levels of serum hCG were found to be over 40,000 mIU/ml in 8 patients, surprisingly, brain metastases could occur even in 2 patients (KAD and RDJ) whose serum hCG levels were extremely low. Only 3 patients had abnormal serum : CSF of hCG concentration (less than 60:1) and one (VAP) occurred before identification of the cerebral lesions. All cytologic studies were negative for malignant cells.

Five patients had received 2 or more chemotherapeutic agents prior to the start of treatment, four of them developed brain metastases later. The average WHO score for all patients was 15.2 (range 7-22).

Sites of metastases are shown in Table 2. Cerebral metastases were likely to occcur equally in both hemispheres, more common at the parietal lobes (5 patients). The other sites were found in equal number of three. Thir-

Table 2 Sites of metastases

Patients	Site of cerebral lesions	Lung	Liver	Others
LFT	Brain stem	-	-	-
CTM	Lt. occipital	+	-	Pelvis, rt. ureter, Scalp, breast
KAD	Rt. parietal	+	-	-
VAP	Brain stem	+	-	-
MJH	Lt. frontal	+	-	-
JFR	Rt. & Lt. occipital	+	-	Pelvis
GD	Rt. parietal	+	-	-
PMG	Lt. parietal	+	+	Spleen, retroperitoneal lymph nodes
TKT	Lt. parietal & Rt. cerebellum	+	-	-
RUN	Rt & Lt cerebellum	+	+	Rt. kidney, adrenal gland, colon, retroperitoneal lymph nodes
RDJ	Brain stem	+	-	-
MLS	Lt. parietal	+	+	Small bowel (jejunum & ileum)
KPC	Rt. frontal	+	-	Rt. kidney
SKS	Rt. frontal	+	+	Rt. kidney, spleen

teen of fourteen patients(93%) were accompanied by pulmonary metastases. Furthermore, there were 7 patients in whom metastases were identified elsewhere, such as liver (4), kidney (3), spleen (2), pelvis (2), retroperitoneal lymph nodes (2), ureter, adrenal gland, scalp, breast, colon and small bowel.

Discussion

In the most recent years, several reports concerning brain metastases of choriocarcinoma from many cancer centers have been presented through various journals amidst, controversial points of view about prevention and early diagnosis, either prophylactic intrathecal methotrexate or serum : CSF hCG ratio, even the classification and staging system.

In our instiute, various diagnostic procedures and therapeutic regimens have been utilized according to the patient's conditions and advancement of medical care. The total number of patients is small, however, we have learned some interesting and valuable points from them. The incidence of choriocarcinoma patients with brain metastases is only 5.3% of total patients diagnosed GTT, which is less than those reported from other institutes[1,9-11]. Fifty per cent of our

patients were referred from elsewhere.

All but one patient (93%) were associated with pulmonary metastases. Reports from other series revealed that 92-100% of choriocarcinoma with brain metastases were preceded by pulmonary metastases[3,4,6,9,10,12,13]. In fact, metastatic pathway of choriocarcinoma to the central nervous system is likely to originate from pulmonary deposits, but some patients may present without any evidence of pulmonary lesions. This finding, however, depends upon sensitivity of pulmonary investigation. Occult pulmonary metastases are frequently identified on CT scanning of the lungs in patients with normal chest radiograph. Approximately 40% of nonmetastatic GTT patients had pulmonary micrometastases detected by CT scanning which were not found by routine chest x-ray[14]. As a result, it would be reasonable to consider screening all patients diagnosed GTT for occult metastases with CT scanning of the lungs.

We found abnormal serum : CSF hCG ratio (<60) in only 3 of our patients. Bagshawe and Harland[3] originally reported that 29 of 33 patients with brain metastases had positive results before any other clinical signs became abnormal[3]. However, Goldstein and Berkowitz[11] had found both false positive and false negative results from their investigation. They concluded that unless corroborated by clinical or radiographic data, or both, a single abnormal serum : CSF hCG ratio is insufficient to diagnose brain involvement. Weed and Hammond[15] suggested that its potential usefulness may be in the asymptomatic patients who have lung metastases, normal brain scans, and persistently elevated hCG titers. We think that it may benefit some cases of negative brain imaging procedures and should be used supplementary to other investigations. Positive results should arouse the suspicion of cerebral involvement. Every effort, utilizing highly sensitive methods, must be made to rule out or detect early cerebral metastases. Although CT scanning is documented to be more sensitive than the isotope scan in detecting cerebral metastases (95% vs 71%), it may be, however, initially negative in patients with asymptomatic metastases[3]. Currently, MRI, the most recent noninvasive diagnostic innovation has been reported to be more sensitive than CT scanning in detecting small metastatic foci[16,18]. In the future, MRI may replace CT scan for brain imaging and play a significant role in detecting occult cerebral metastases.

GTT patients with brain metastases are classified as a high-risk group according to Hammond clinical classification system and are also in stage IV when classified by anatomic staging system of FIGO Cancer Committee[19]. We categorized the patients based on WHO prognostic scoring system and found that all 14 patients fell into the high-risk group if ABO blood group was not considered. Analyzing the experience at the New England Trophoblastic Disease Center, DuBeshter et al[20] found that the

WHO scoring system was more effective than traditional criteria in predicting which patients required intensive combination chemotherapy initially. The WHO score seems to be a very important factor predictor of treatment outcome[8,21,22].

For early detection of brain metastases, all patients diagnosed persistent GTT should be categorized according to WHO prognostic score to identify high-risk group and undergo through metastatic work up. Any low-risk patients, patients with pulmonary metastases and all high-risk patients should receive careful metastatic search for brain involvement by highly sensitive methods including CT scanning or MRI. Supplemental serum : CSF hCG measurement may be individually considered in case of negative metastatic work-up.

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The Effect of a Thromboxane Synthetase Inhibitor, Sodium Ozagrel, on Pregnancy-induced Hypertension

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Abstract: *Synthetic selective thromboxane synthetase inhibitor, sodium ozagrel was administered intravenously to 5 pre-eclamptic pregant women at 29 - 35 weeks gestation. Dose of ozagrel was 80 - 160 mg/day and was infused continuously for 8 - 22 days.*

Systolic and diastolic blood pressure decreased gradually and became stable in all patients after administration of ozagrel. Clinical signs improved and proteinuria decreased. In one patient, blood pressure became stable at first but then became uncontrollable. Caesarean section was performed in 2 patients because of fetal distress and severe hypertension. All neonates were well-developed without any complications.

Thromboxane B_2 to 6-keto-$PGF_{1\alpha}$ ratio and 2,3-dinor-thromboxane B_2 values decreased after administration of sodium ozagrel.

Sodium ozagrel seems likely to be useful and effective for stabilization of blood pressure and prevention of exacerbation. The results of this initial clinical study are very encouraging with respect to the treatment and prevention of pregnancy-induced hypertension. (Thai J obstet Gynaecol 1991; 3:39-44.)

Key words: pregnancy-induced hypertension, thromboxane, prostacyclin, thromboxane synthetase, sodium ozagrel

Pregnancy-induced hypertension (PIH) has been suggested to be caused by an impaired production of prostacyclin (PGI_2) and increased generation of thromboxane A_2 (TXA_2)[1,2]. PGI_2 is a potent vasodilator and an inhibitor of platelet aggregation and of uterine contractility. On the other hand, TXA_2 increases vasoconstriction, stimulates platelet aggregation, increases uterine contractility and decreases uterine blood flow. It is widely accepted that PGI_2 and TXA_2 involved in the control mechanisms of microcirculation and blood pressure. Therefore, the higher level of PGI_2 in peripheral blood rather than TXA_2 is required to prevent or treat PIH.

Sodium ozagrel (Sodium (E)-3-[p-(1H-immidazole-1-yl methyl) phenyl]-2-propenoate, Ono Pharmaceutical Co., Ltd., Osaka, Kissei Pharmaceutical Co., Ltd., Matsumoto), one of the stable imidazole derivatives, was synthesized as a potent selective inhibitor of thromboxane synthetase in Japan in 1978. In Japan, sodium ozagrel has been used clinically for the treatment of vasospasm of cerebral vascular diseases and coronary diseases since it inhibits vasospasms and platelet aggregations. The reason for the study was to investigate whether sodium ozagrel can be used as an alternative for the treatment of PIH.

Materials and Methods

After informed consent was obtained, sodium ozagrel (80- 160 mg/day) was administered intravenously to five pre-eclamptic pregnant women at 29-35 weeks gestation for 8-22 days. Three cases had a family history of hypertension and one case had a past history of PIH. In four cases, combined therapy of other antihypertensive drugs was needed. Clinical signs, fetal growth, fetal heart rate, placental function tests, and renal function test were monitored during the treatment.

Thromboxane B_2 (TXB_2), 6-keto-$PGF_{1\alpha}$ in blood and 2,3-dinor-TXB_2 in urine were measured by RIA techniques[3,4].

Results

The clinical characteristics of the patients with PIH are shown in Table 1.

Ozagrel decreased and stabilized systolic and diastolic blood pressure gradually in all patients and hypertension recurred by cessation of ozagrel (Figs.1-3). Clinical signs improved in all cases and protein in urine decreased in four out of five patients. In one patient, blood pressure became stable at first but then became uncontrollable. In this case, caesarean section was performed because of uncontrollable maternal hypertension. In the other case, caesarean section was performed because of fetal distress without maternal hypertension. There were three vaginal deliveries. All neonates were well-developed without any complications (Table 2).

Table 1 Clinical characteristics of 5 patients with PIH and treatment

Case	Age G-P	Obstetrical history Family history	Gestational weeks at onset of PIH Complications	Gestational weeks at onset of the treatment Dose of Sodium Ozagrel (mg/day) × duration	Other drugs	Side effects
1	38 5-2	Abortion 3× 1978. NVD(40W) PIH, IUGR 1988. IUFD(36W) PIH, IUGR	31W(HPE) liver dysfunction (33W)	31W 80mg × 22days	labetalol 300mg ⇒ 400mg nifedipine 10mg ⇒ 20mg	elevated liver enzyme
2	43 4-4	NVD 4× Hypertension (mother, father)	31W(HPE)	34W 80mg × 18days	labetalol 300mg nifedipine 20mg ⇒ 30mg	(−)
3	26 1-0	Abortion 1× Hypertension (father)	25W(HPE) premature labor oligohydramnios	29W 160mg × 8days	labetalol 300mg ⇒ 450mg	(−)
4	35 0-0	Hypertension (mother)	34W(HPe) GDM premature labor	35W 160mg × 9days	diltiazol 200mg	(−)
5	29 2-0	Abortion 2×	20W(HPE) renal dysfunction (35W) premature labor oligohydramnios	35W 160mg × 8days	albumin 5 g furosemide 40mg	(−)

N-acetyl-β-D-glucosaminidase (NAG) values in urine, which represent the function of the renal tubules, also decreased in three out of four measured cases after administration of sodium ozagrel, coincident with the

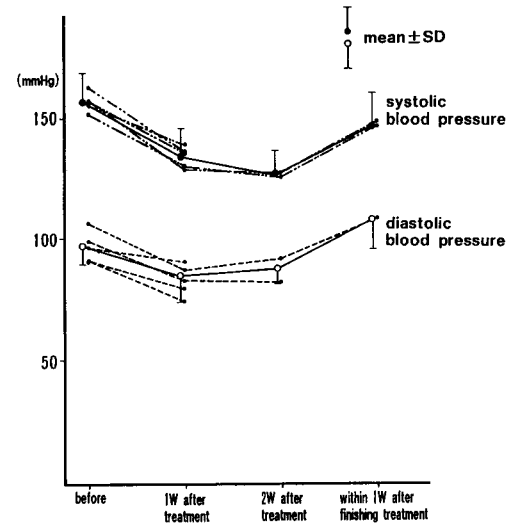

Fig. 1 Changes of blood pressure with ozagrel therapy.

Table 2 Clinical outcome and neonatal course

Case	Gestational weeks at delivery	Mode of delivery	Blood loss	Weight and pathology of the placenta	Neonatal body weight and findings	Neonatal course
1	37W 1D	V	118 ml	450g small infarcts	1,988g, ♂ (LFD) Apgar 9	Hyper-bilirubinemia. good
2	37W 4D	V	204 ml	360g small infarcts	2,536g, ♀ (AFD) Apgar 8	good
3	30W 3D	C/S (fetal distress, preeclampsia)	230 ml	240g premature placenta with scattered small infarcts	1,112g, ♂ (LFD) Apgar 5	Hyper-bilirubinemia. PDA good
4	36W 3D	Forceps (fetal distress)	1095 ml	750g no infarct	3,412g, ♀ (HFD) Apgar 9	good
5	36W 3D	C/S (fetal distress)	680 ml	310g multifocal infarcts	1,694g, ♂ (LFD) Apgar 8	good

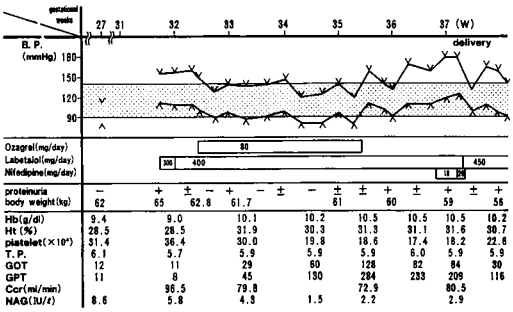

Fig. 2 Course of clinical measurements, laboratory test values and treatment in case No.1.

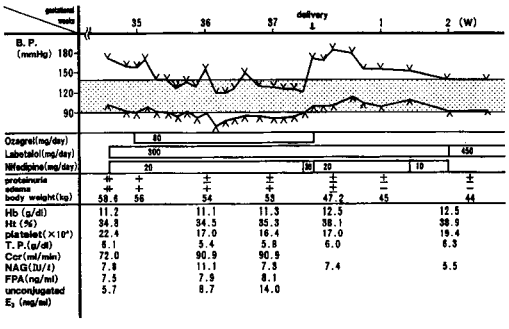

Fig. 3 Course of clinical measurements, laboratory test values and treatment in case No.2.

disappearance of proteinuria, but creatinine clearance values did not change (Fig. 4). Human placental lactogen and unconjugated estriol values in plasma in case 1 and 2 increased within normal range during treatment but after cessation of treatment those values, especially unconjugated estriol value, decreased (Figs. 3,5).

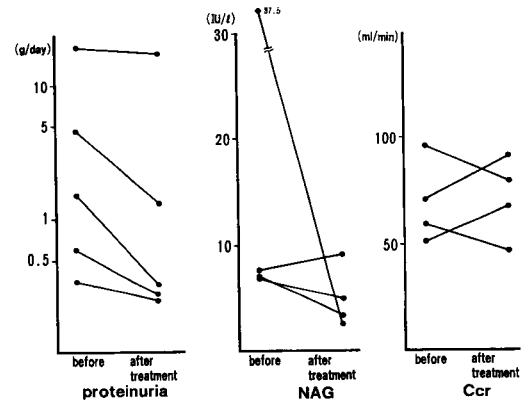

Fig. 4 Changes of the renal function test values after administration of sodium ozagrel.

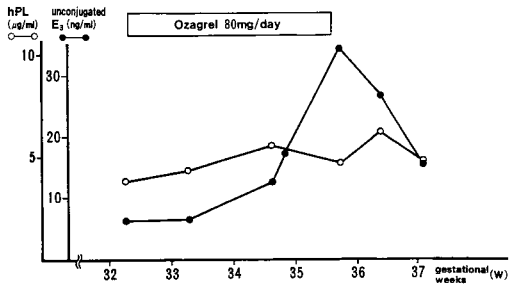

Fig. 5 Changes of hPL and unconjugated estriol values in case No.1 by administration of sodium ozagrel.

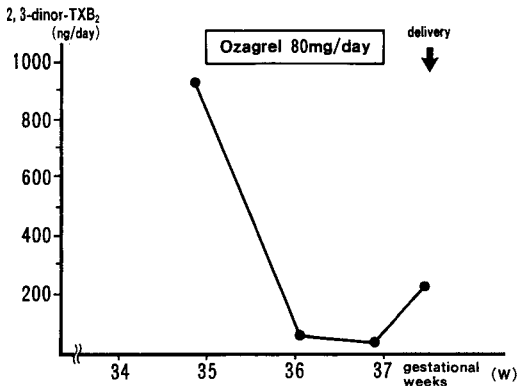

Fig. 7 Changes of urinary 2, 3-dinor-TXB_2 values in case No.2 by administration of sodium ozagrel, 80 mg/day.

TXB_2 concentrations in plasma and TXB_2/6-keto-$PGF_{1\alpha}$ ratio decreased after treatment, but changes of 6-keto-$PGF_{1\alpha}$ concentrations were not noted (Fig. 6). Urinary 2,3-dinor-TXB_2 concentrations, that is a metabolite of thromboxane, decreased after treatment (Fig. 7).

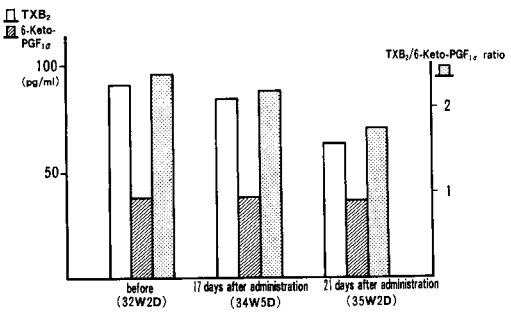

Fig. 6 Plasma concentrations TXB_2 and 6-keto-$PGF_{1\alpha}$ in case No.1 before and after administration of sodium ozagrel.

Discussion

In 1977, Moncada[5] reported that imidazole derivatives had an inhibiting effect on thromboxane synthetase. Needleman[6] reported that imidazole promoted prostacyclin production in the arterial wall. Sodium ozagrel, one of the imidazole derivatives, was synthesized in 1978 and then manufactured in Japan. Its molecular weight is 250 and its half life is very short, 40 minutes, in blood. So, continuous intravenous infusion is necessary because of its short half life and its rapid secretion into the urine. The pharmacological action of sodium ozagrel is its selective and strong inhibition of thromboxane synthetase[7], and it does not work on cyclooxygenase, prostacyclin synthetase and lipoxygenase[8]. Its physiological action is the inhibition of vasospasms and platelet aggregations. Increasing production of PGI_2 is explained by the increased PGH_2, a precursor of PGI_2, by inhibition of TX synthetase (Fig.8).

In patients with cerebral vascular diseases and coronary diseases, plasma TXB_2 and 6-keto-$PGF_{1\alpha}$ concentrations during treatment of sodium ozagrel have been reported[9]. TXB_2 levels decreased and 6-keto-$PGF_{1\alpha}$ lev-

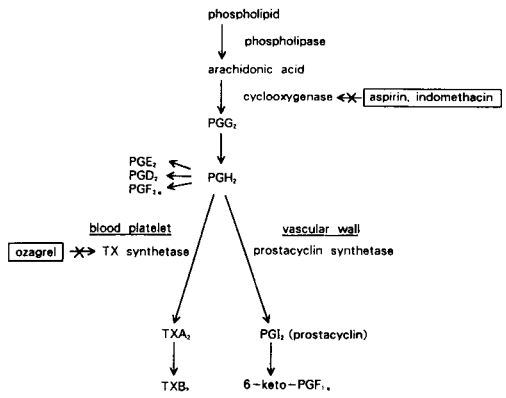

Fig. 8 A metabolic pathway of arachidonic acid and inhibitors of the enzyme.

els were increased significantly after administration of ozagrel[8,9]. In cases of pre-eclampsia, plasma TXB_2 and urinary TXB_2 metabolite concentrations markedly decreased but 6-keto-$PGF_{1\alpha}$ levels did not change. It was reported that in pre-eclampsia, activity of prostacyclin synthetase were lowered, compared with thromboxane synthetase activity, because the endothelial cells on the vascular wall had been damaged[10]. It seems likely that prostacyclin production in the vascular wall is restricted in pre-eclampsia, and clinical application of sodium ozagrel for severe PIH seems to be ineffective, since blood pressure was uncontrollable in one case in spite of administration of ozagrel. However, the study showed that sodium ozagrel has an inhibiting effect of thromboxane synthetase *in vivo* as well as *in vitro*.

Sodium ozagrel was also effective not only for improvement of hypertension but also for improvement and prevention of exacerbation of proteinuria, function of the renal tubules and placental function. It seems probable that ozagrel affects the renal tubules rather than the glomerulus. A mechanism of ozagrel action is still uncertain *in vivo*, but it might improve the renal and placental microcirculations by prevention of vasospasm and platelet aggregation.

Sodium ozagrel in maternal circulation is easily transferred into the fetus because of its small molecules. But there are no adverse effects in neonates because of its rapid metabolic clearance. In some animal experiments, fetal anomaly and death were not reported.

Sodium ozagrel seems likely to be useful and effective for stabilization of blood pressure and improvement of microcirculation and prevention of exacerbation. Results of this initial clinical study are encouraging enough to introduce the new treatment and prevention of PIH.

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Bacterial Interference by Group B Streptococci with Aerobic and Anaerobic Genital Tract Streptococci and Nonstreptococcal Aerobic Bacteria of the Female Genital Tract

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Abstract : *Target groups composed of 10 isolates of each of the following: S. viridans, non-hemolytic streptococci not group B or D, group A streptococci, group B streptococci, peptostreptococci, coagulase-negative staphylococci, S. aureus, G. vaginalis, E. coli, 9 enterococci, 9 group C or G streptococci; 7 lactobacilli and 7 diphtheroids were tested for inhibition by a test panel of a group of 10 or 41 group B streptococci (GBS). The GBS did not inhibit the growth of E. coli, coagulase negative staphylococci or S. aureus. They uniformly inhibited the groups A, B, C and G streptococci, lactobacilli and G. vaginalis. One isolate of diphtheroids was inhibited by 37 of 41 GBS; the other six isolates were uniformly inhibited. Variable inhibition was observed with the viridans streptococci, non-group B or D streptococci and enterococci; however, inhibition or noninhibition was uniform for a given isolate against the entire group B streptococcal test panel. The 23 isolates of the group B streptococci from neonates or adults with septicemia, did not differ from isolates from patients with only local disease in their ability to inhibit other species tested. The group B streptococci uniformly inhibited the aerobic lactobacilli, diphtheroids and G. vaginalis but had no effect on the coagulase-negative staphylococci, S. aureus or E. coli. These studies suggest that group B streptococci may be significant regulators of the female genital tract bacterial flora. (Thai J Obstet Gynaecol 1991;3:.45-52.)*

Key words : bacterial interference, group B streptococci, female genital tract

When quantitative and qualitative bacteriological studies are performed on normal cervical and vaginal vault bacterial flora, the dominant aerobic groups of bacteria are the lactobacilli, diphtheroids, staphylococci and streptococci[1-6]. The dominant anaerobic groups are composed of the

Gram positive bacilli (which include lactobacilli, eubacteria and clostridia), peptostreptococci and the Bacteroidaceae. Group B streptococci (GBS) constitute a potentially important subgroup within the streptococci. Not only is it a frequent constituent of normal genital tract bacterial flora, but it is also the most frequent monomicrobial cause of disease in both parturitional gravida and neonate[7-10].

This paper analyzes the ability of 23 isolates of GBS obtained from septicemic patients and 18 isolates of GBS from alternate sites to inhibit other constituents of the female genital tract bacterial flora.

Materials and Methods

Strains

Initially, an inhibitor test panel of 41 isolates of group B streptococci was examined. These inhibitor isolates were tested against target cultures of other streptococci and aerobic bacteria common in the vaginal flora. Twenty-three of the 41 group B streptococci were obtained from blood cultures. Of the 23 isolates from septicemic patients, 15 were from infants with early onset and late onset group B streptococcal disease. The non-septicemic isolates were isolates from random genital tract or wound cultures. Because of the uniformity of inhibition observed within the entire 41 isolates of group B streptococci, the inhibitor test panel was reduced to 10 isolates of group B streptococci for certain target strains. Of these 10 isolates, 5 isolates were derived from cases of early onset neonatal septicemia, and 5 were incidental isolates from genital tract cultures. Internal controls were run with each test.

The target cultures included 10 viridans streptococci, 10 group B streptococci, 10 non-hemolytic streptococci not group B or D, 10 group A streptococci, 9 group C or G streptococci, 10 peptostreptococci, 9 enterococci, 10 coagulase-negative staphylococci, 10 S. aureus, 10 E. coli, 7 lactobacilli, 7 diphtheroids, and 10 G. vaginalis.

Media

Tripticase Soy Agar (TSA, Baltimore Biological Laboratories, Baltimore, Maryland) was used for both layers in the overlay procedure. Organisms were maintained on Tripticase Soy Agar supplemented with 5% sheep blood (BAP, Scott Laboratories, Fiskeville, Rhode Island).

Overlay assay

Overlays performed were a modification of the technique described by Fredericq[11] and modified by Crowe, Sanders and Longley[12] and Murray and Rosenblatt[13]. Each strain of group B streptococcus was inoculated onto a one square centimeter area of a 15 ml TSA plate. The assays were performed in duplicate. Four strains per plate were tested. Each plate was rerun in a separate test under code.

The organisms were incubated for 18-24 hours in 10% carbon dioxide at 35° C. They were overlaid with 7.5 ml molten TSA which was allowed to solidify. The target strain was then inoculated onto the top of the fresh TSA in the following manner.

A 0.4 OD at 450 nm of the target strain was made in physiological saline. A 1:10 dilution was made in saline, and a 2 ml quantity of this was inoculated onto the freshly overlaid plate. The excess was siphoned off, and the plates were incubated for 24 hours at 35° C in 10% carbon dioxide. After incubation, the assays were examined for inhibition or no inhibition of growth of the target strain.

Results

Streptococcus viridans

Seven isolates were inhibited by all group B streptococci examined in 101 tests (Table 1). Three isolates were not inhibited by group B streptococcal test panel. When inhibition was observed, the phenomena was uniform for the entire 10 or 41 group B streptococcal panel.

Non-hemolytic streptococci, not group B or D

Of the 10 target isolates of the non-hemolytic streptococci, not group B or D, 9 isolates were inhibited while 1 isolate was not. Comparable inhibition was produced by all of group B streptococci tested.

Enterococci

Of 9 isolates of enterococci, only 1 isolate was inhibited by group B streptococci while the remaining 8 were not (Talbe 1). The results were uniform for both inhibition and noninhibition for the entire group B streptococcal panel, however, the degree of inhibition did vary from isolate to isolate. When 5 strains of the enterococci were used as the inhibitor strain, all 10 isolates of group B streptococci isolates tested in 50 challenge experiments were inhibited.

Group A streptococci

For the 10 target isolates of group A streptococci, all were inhibited by group B streptococci in the 193 challenge experiments (Table 1).

Group B streptococci

For the 10 target isolates of group B streptococci, inhibition was total in 193 challenge experiments (Table 1).

Group C or G streptococci

For the 9 challenge isolates of group C (7) or group G (2) streptococci, inhibition was total in the 183 challenge experiments (Table 1).

Peptostreptococci

Of the 10 peptostreptococci, 7 challenge isolates were inhibited completely. Three of the 10 were uninhibi-

Table 1 Inhibition of target bacteria by group B streptococci isolates

Taret bacteria	Number of strains tested	Number of observations	Number of strains / Number of observations	
			Inhibited	Non-inhibited
Viridans streptococci	10	193	7/101	3/92
Non-hemolytic streptococci-not group B or D	10	193	9/183	1/10
Enterococci group A	9	276	1/41	8/235
Streptococci group B	10	193	10/193	0
Streptococci group C (7)	10	193	10/193	0
or G (2)	9	183	10/183	0
Streptococci Pepto-streptococci	10	193	7/132	3/61
Coagulase-negative staphylococci	10	193	0	10/193
Staphylococcus aureus	10	193	0	10/193
Escherichia coli	10	193	0	10/193
Lactobacilli	7	163	7/163	0
Diphtheroids	7	194	7/190*	0/4
Gardnerella vaginalis	10	19	10/193	0

*4 of group B streptococci in the panel of one isolate were not inhibitory.

ted. The target isolates exhibited a uniform pattern of inhibition or noninhibition by group B streptococci. The presence or absence of inhibition for the individual species of the peptostreptococci is listed in Table 2.

Coagulase-negative staphylococci

None of the 10 target isolates tested in 193 individual challenge experiments was inhibited by group B streptococci (Table 1). When five

strains of coagulase-negative staphylococci were used as the inhibitors, all 10 group B streptococcal isolates tested in 50 challenge experiments were inhibited.

Staphylococcus aureus

None of the 10 target isolates tested in 193 individual challenge experiments was inhibited (Table 1). When 5 strains of S. aureus were used as the inhibitor cultures, none of 10 group B streptococci isolates tested in 50 challenge experiments was inhibited.

Escherichia coli

None of the 10 isolates tested in 255 individual challenge experiments was inhibited by group B streptococci (Table 1).

Lactobacilli

All 7 target isolates tested in 163 individual challenge experiments were inhibited (Table 1).

Diphtheroids

All 7 target isolates tested individually were inhibited. One isolate had a variable pattern of inhibition such that of the 194 individual experiments, 190 showed inhibition (Table 1).

Gardnerella vaginalis

All 10 target isolates tested in 193 individual challenge experiments were inhibited (Table 1).

Table 2 In vitro bacterial interference by the group B stereptococci on strains of peptostreptococci

Strain of peptostreptococci	Number of test strains of group B streptococci	Percentage of inhibition
P. tetradius	10	100
P. tetradius	10	0
P. anaerobius	41	100
P. anaerobius	10	100
P. anaerobius	10	100
P. micros	41	100
P. micros	10	0
P. asaccharolyticus	41	0
p. asaccharolyticus	10	100
P. asaccharolyticus	10	100

Discussion

The initial concept of bacterial interference eminated from the observations of Pasteur and Joubert[14]. They noted that some urine cultures of B. anthracis would die if they became contaminated with other bacteria.

The mechanisms by which a bacterial species maintains its ecological niche are varied. Inhibitory bacterial products include a wide range of substances: low molecular weight antibiotics, metabolic products, hydrogen peroxide, lytic agents, enzymes, bacteriocins and bacteriophages[15,16].

The ultimate question for group B streptococci is how does a normal constituent of the bacterial flora of the female genital tract become the causative agent of septicemia in mother and neonate[7-9]. As demonstrated in these studies, group B streptococci has the ability *in vitro* to effect bacterial interference. If these mechanisms function *in vivo,* group B streptococci would possess the capability of defending their microbiological niche not only against other group B streptococci but also against other aerobic beta-hemolytic strains (group A, C and G streptococci). This ability appears to be uniform. The uniformity of this effect may be the result of the genetic interrelationship between hemolytic activity and bacterial interference. Brock et al[17] found that, by categorizing strains of S. zymogenes in terms of their hemolytic character, they could demonstrate uniform bacterial interference which was mediated by bacteriocins. There was no variation in the ability to inhibit bacterial replication between septicemic and nonsepticemic isolates of group B streptococci. Group B streptococcal potential for *in vitro* governance over the non-hemolytic streptococci not group B or D gamma hemolysis is significant. The majority of the isolates (95%) exhibit complete inhibition; their impact on the viridans streptococci and peptostreptococci is significantly less. Group B streptococci inhibited other common non-streptococcal Gram-positive aerobic bacteria and G.vaginalis but had no impact on the staphylococci and E. coli.

The importance of the lactobacilli may be more as regulators of the enterococci than major regulators of the female genital tract bacterial florad (FGTBF). De Klerk and Coetzec[18] studied bacterial inhibition by the lactobacilli. Using the supernatants concentrated by ammonium sulfate precipitators, they were able to demonstrate an antibacterial activity which was primarily restricted to certain members of the family Lactobacteriaceae. A significant number of enterococci were inhibited. The antibiotic-like supernatants did not impact on the Enterobacteriaceae or staphylococci. Holmberg and Hallander[19] documented the ability of S. sanguis to inhibit L. acidophilus, L. fermentum and L. casei. Pohonch[20], among others, has similarly demonstrated the ability of the streptococci to inhibit the vaginal lactobacilli.

Statistically, coagulase-negative staphylococci are more frequently present in the FGTBF than S. aureus[1-4,23]. Both coagulase-negative and -positive staphylococci have the ability to inhibit bacterial replication of other bacteria *in vitro*. Prior work by Dajani et al[21] demonstrated the ability of S. aureus to elaborate a bactericidal substance which inhibited groups A, D and G streptococci. Observations derived from clinical disease in which both staphylococci and beta-hemolytic streptococci can be concomitantly isolated from skin lesions have questioned whether the staphylococci invade sites previously infected with a beta-hemolytic organism or whether there is a significant coupling between the 2 groups of Gram-positive bacteria[22-24]. The ability of a given strain of the Enterobacteriaceae to inhibit other members of the family has been well documented[16]. The predominance of a strain of E. coli as the principal Enterobacteriaceae in FGTBF may be selected by the Bacteroidaceae. Murray and Rosenblatt[13] demonstrated that B. melaninogenicus, B. fragilis and B. oralis, while possessing significant ability to inhibit E. cloacae, E. aerogenes, Klebsiella species and S. marcescens, were ineffective against E. coli and M. morganii. The Bacteroidaceae did have moderate inhibitor activity for coagulase-negtive staphylococci but almost no activity against S. aureus. In their paper, Fusobacteria and L. fermentum had little inhibitory effect on either Gram-negative or Gram-positive bacteria. Interspecies governance among the Enterobacteriaceae is probably mediated by bacteriocins, however, the predominace of E. coli and P. mirabilis may be a direct function of their resistance to bacterial inhibition by the Bacteroidaceae and selected peptostreptococci.

Demostration of the *in vitro* ability of group B streptococci to inhibit all group A, B, C and G streptococci, lactobacilli, diphtheroids and G. vaginalis as well as most non-hemolytic streptococci not group B or D and viridans streptococci, infers that this organism may be a significant regulator of the female genital tract bacterial flora should those phenomena function *in vivo*.

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Microbial Findings in Amniotic Fluid Following Serial Amniocentesis

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Abstract : *In order to determine the reliability of amniocentesis (ACT) and its influence on intraamniotic infection (IAI) development, we performed a study of sixty complete microbiological examinations of amniotic fluid specimens obtained by serial ACT. In one case during the second procedure, E. coli was discovered and in the other, during the third ACT, Candida albicans was presented. Neither spontaneus abortion nor preterm labour were provoked by the procedure. We can conclude that ACT has been confirmed as a safe and successful intrauterine intervention if it is made in the proper way. (Thai J Obstet Gynaecol 1991; 3:53-56.)*

Key words: intraamniotic infection, microbiological findings, serial amniocentesis

Fetus in utero is in a sterile environment, protected from contact with most microorganisms. It is regarded as a compromised host, because its specific and nonspecific immune mechanisms are deficient[1,2].

Infections of feto-maternal compartment are both common and potentially life-threatening to the mother and her fetus. These infections can range from mild to severe or may even go unnoticed[3,4]. With the onset of labour or with membranes rupture bacteria from the lower genital tract commonly ascend into the amniotic cavity. This is the most common pathway for intraamniotic infection (IAI) development[5]. Occasional cases of IAI with intact membranes and without labour support a presumed hematogenous or transplacental route of infection[6]. Less commonly, IAI may develop as a consequence of obstetric procedures such as cervical cerclage, diagnostic amniocentesis (ACT) and intrauterine transfusion[7,8].

The aim of this study was to investigate the influence of serial amniocentesis on IAI development.

Materials and Methods

This study comprised 16 patients in whom amniocentesis was

performed twice and 6 with three subsequent interventions. In two gravidas, serial collections of the amniotic fluid specimens were made five times. Specimens for analysis were taken at intervals of 2-3 weeks. First, ultrasound examination was performed to select a site on the maternal abdomen for insertion of the needle into the amniotic sac. If possible, insertion through the placenta was avoided. After a site was chosen and marked, the abdomen was cleaned with an antiseptic solution and, under real-time ultrasound guidance, a 20- or 22-gauge spinal needle was inserted transabdominally into the amniotic sac. After the stylet was removed, the first amniotic fluid specimen was aspirated into the syringe and used for microbial testing, and the second specimen was sent for the ACT analysis. A specimen from the first syringe was cultivated in blood and endoagar and tyoglicolate medium for microbial propagation, and then incubated in thermostate at 37° C over 24 hours. The material was resume to allow further analysis of microbes.

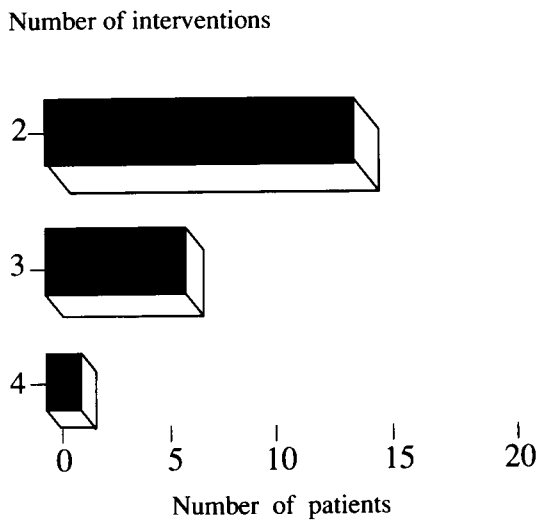

Fig. 1 Serial amniocentesis in studied patients .

Results

Sixty complete microbiological examinations of the amniotic fluid specimens obtained by serial ACT were performed. Amniotic fluid was collected twice in 16 patients, three times in six and in two gravidas as much as five times (Figure 1). In one specimen obtained by the second procedure E. coli was present,while in the other, after the third ACT, Candida albicans was isolated. No spontaneous abortion or preterm labour were provoked by ACT.

Discussion

There are few well-designed prospective studies that prove the beneficial effects of ultrasonography before ACT[9,10]. The ultrasound is useful during the procedure for the following purposes of a) to confirm fetal viability and gestational age and b) to determine placental location in order to avoid blood-contaminated specimens[8]. According to the literatures clinical evidence of IAI is seen in only about 1% of pregnancies and is associated with an increase in maternal and perinatal morbidity and mortality[6,11,12]. IAI in fact, occurs predominantly in patients with ruptured membranes and after the onset of

labour. This ascending route of infection from the lower genital tract is the most common mode of infection[4, 13, 14]. There are, however, many reported cases of documented IAI in patients without labour or rupture of membranes[2,8,15]. Miller et al[17] reported on 23 "selected" afebrile asymptomatic women in labour before 36 weeks and found ten positive amniotic fluid cultures (43%), five on primary plating and five in broth only. Bobitt et al[16] found six positive amniotic fluid cultures (21%) among 29 apparently afebrile asymptomatic women in preterm labour with intact membranes before 35 weeks. Traditionally, the mode of infection in these patients is thought to be hematogenous or transplacental. Recently, it has been recognized that IAI, especially subclinical, can occur secondary to ascending infection in the face of intact membranes[6]. In recent investigations, using ACT in the evaluation of preterm labour, it has been found that 3% to 26% of gravidas were culture-positive with intact membranes[5,6,14]. In our study, in two cases the presence of microbes in amniotic fluid specimens were observed. Numerous investigations suggest that ACT leads to IAI in about 0-1% of patients[8]. However, no one has studied the influence of serial amniocentesis on the development of this pathologic state. Our study revealed the presence of microorganisms in only two amniotic fluid specimens after the second and third procedure.

In conclusion, ACT under ultrasound control has been confirmed as a safe and successful intervention without risk for IAI if it is done in the proper way.

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Antepartum Diagnosis of Potter Syndrome

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Abstract : *Potter syndrome (bilateral renal agenesis) is one of the rare lethal congenital malformations. This report describes a case of Potter syndrome diagnosed antenatally by ultrasonography and fetal pyelography. In cases of IUGR with oligohydramnios, the fetal well-being should be intensively assessed by ultrasonography, ultrasound-guided umbilical cord blood sampling and nonstress test. (Thai J Obstet Gynaecol 1991; 3:57-61.)*

Key words: Potter syndrome, antepartum diagnosis, fetal pyelography, oligohydramnios

The impact of the diagnostic application of ultrasound and its guided fetal cord blood sampling for the identification of various fetal anomalies has been momentous. Antenatal recognition of a lethal malformation would be helpful in deciding the most appropriate mode of delivery and in reducing the psychosomatic burden of parents for an unfavorable outcome.

Potter syndrome is a congenital malformation accompanied by bilateral renal agenesis, lung hypoplasia, characteristic facies and other abnormalities[1,2]. It is also often associated with oligohydramnios, intrauterine growth retardation and breech presentation. Since these infants die of renal or respiratory failure shortly after birth, the accurate antenatal diagnosis of Potter syndrome is of importance in order to avoid unnecessary caesarean section.

The present report shows a case of Potter syndrome diagnosed antenatally by ultrasonography, fetal pyelography and the analysis of umbilical cord blood.

Case Report

The patient was a 34-year-old, married woman, para 2-0-2-2, referred to the hospital because of retarded growth of the fetus at 29 weeks of

pregnancy. The family history was noncontributory. The prenatal course until then was uneventful. All blood analysis was normal except 75g GTT. GTT at 29 weeks of gestation showed 120, 245 and 231 mg/dl. An ultrasound examination revealed no detectable amount of amniotic fluid and an extremely growth-retarded fetus with scaphoid head, bell-shaped thorax and no demonstrable kidney (Figs. 1,2). The fetal bladder did not appear to fill during more than one hour of observation. Moreover, maternal administration of furosemide 20 mg failed to cause the filling of the fetal renal pelvis and the bladder with urine (Fig. 2), but nonstress testing was reactive (Fig. 3). Unconjugated estriol and hPL values were 5.8 ng/ml and 2.0 μU/ml, respectively, Repeated examination by ultrasonography did not demonstrate the fetal kidney. At 31 weeks, ultrasound-guided sampling of umbilical cord blood and fetal intravenous pyelography were performed to make an accurate diagnosis. The results of fetal blood analysis were as follows; WBCx 7800/mm^3, RBC 350 x 10^4/ mm^3, Hb 11.8 g/dl, Ht 36.0%, platelet count 178x10^3/mm^3, total protein 3.7g/dl, albumin 2.6 g/dl. Fetal anemia and hypoproteinemia were remarkable. Electrolytes and blood gas analysis were within normal range: Na 136 mEq/l, K 4.6 mEq/l, Cl 105 mEq/l, BUN 10 mg/dl, creatinine 0.6 mg/dl, pH 7.373, PCO_2 43.1 mmHg, PO_2 20.2 mmHg, HCO_3 25.0 mEq/l. Fetal nephrogram and pyelogram, administered with 5 ml of 60% urographin intravenously, did not contrast. On the other hand, maternal pyelogram was obtained in spite of fetal administration of the contrast medium.

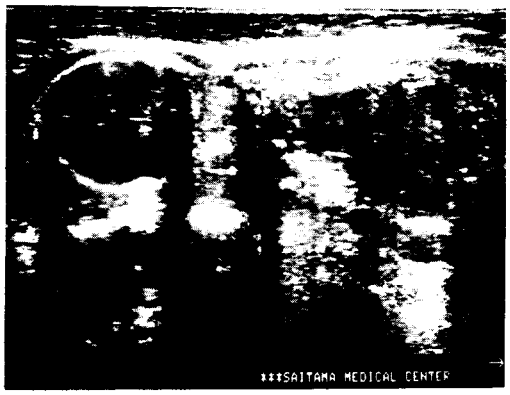

Fig. 1 Ultrasound picture of the fetus in utero (29th wk). Sagittal section of the fetus at the level of the head and thorax. Fetus is touched to the uterine wall, caused by anhydramnios. Bell-shaped thorax is characteristic.

Fig. 2 Ultrasound pictures of the fetal head (left) and trunk (right). Transverse section of the fetus at the level of the kidney, which is not demonstrable after the maternal administration of furosemide. An extremely growth-retarded fetus with scaphoid head is observed.

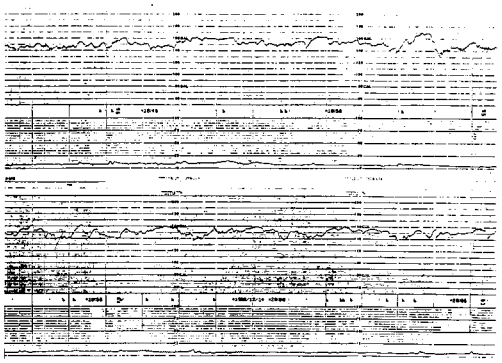

Fig. 3 Fetal cardiotocogram. Nonstress testing is reactive at 30 weeks of gestation.

A diagnosis of Potter syndrome was made by the findings of ultrasonography and fetal pyelography.

The patient went into spontaneous labour two days later and had a stillbirth in breech presentation. The infant weighed 1062 g. No amniotic fluid was recognized. The placenta weighed 200 g. The Potter facies with prominent epicanthic folds, flattened nose, low set ears and receding chin were noted. There were bowing legs with hypogenesis of the left foot, atresia ani and single umbilical artery. External genitalia was defective (Fig. 4). At autopsy, the infant was proved to be male with agenesis of bilateral kidneys. Hypoplastic lungs, malrotation of the intestine, mesenterica communis and retentio testis were also seen.

Discussion

In 1946, Potter[1] reported twenty instances of bilateral renal agenesis with pulmonary hypoplasia and a characteristic facial expression. Potter syndrome occurs predominantly in males and about once in 2500-3000 births[2,3]. Dry, loose skin, limb abnormalities such as bowed legs and clubbed feet, spade-like hands, ovoid adrenal glands, and urinary tract or genital abnormalities are also usually observed. Oligo- or anhydramnios, intrauterine growth retardation, prematurity and breech presentation are common. It is recognized that similar signs and symptoms are accompanied by the various types of urinary tract abnormalities and are also the outcome of fetal compression due to nonrenal oligohydramnios secondary to prolonged leakage of amniotic fluid, hence the term Potter sequence.

The aetiology is still unknown. The development of Potter syndrome may be due to the consequence of multiple early mesodermal defects which occur mainly sporadically or rarely in autosomal recessive inheritance. In Potter syndrome, limb deformities, flattened ears and nose, and hypoplastic lungs can be attributed to compression secondary to oligohydramnios but the epicanthic fold, the malformation of the tragus and antetragus, the abnormally low slanted position of ears, the changes in subcutaneous tissue, and the increased frequency among males cannot be explained merely due to oligohydramnios.

It has become much easier to detect various fetal malformations by monitoring the fetus by ultrasound equipment with high resolution. Antenatal diagnosis of urinary tract anoma-

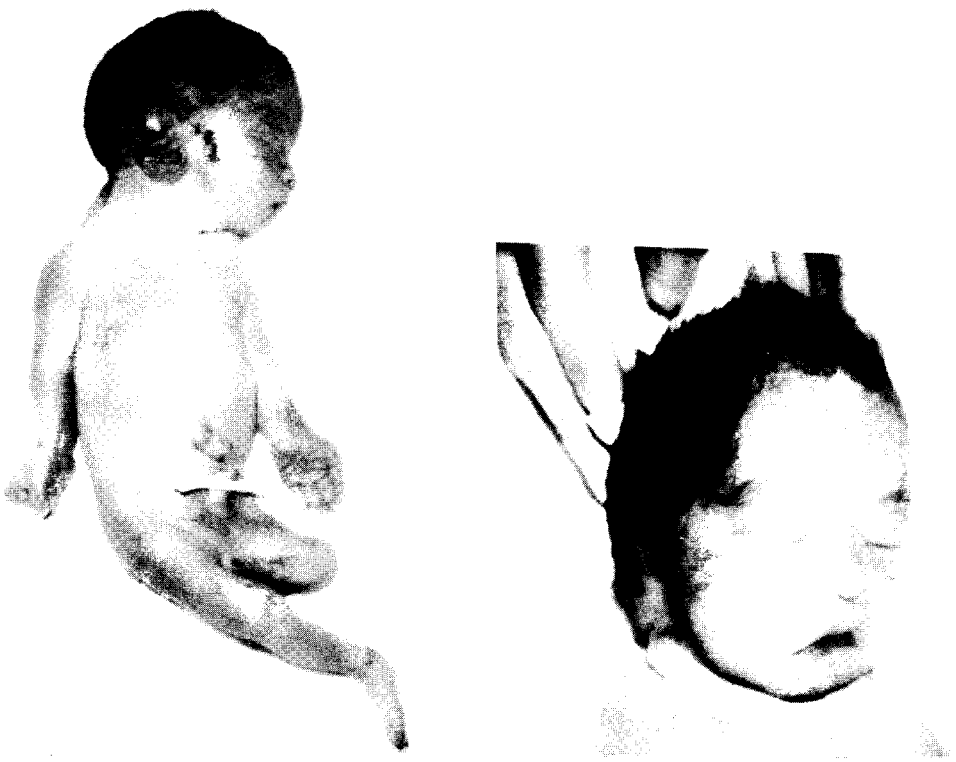

Fig. 4 Photographs of the fetus characteristic of the Potter face and bowing legs with hypogenesis of the left foot.

lies such as cystic kidney and obstuctive uropathies, has also been reported[3,6]. In the normal fetus the kidneys and urinary bladder can be easily identified after the 16th-20th week of gestation[5,7,8]. In addition to this, changes in bladder volume by urine can be observed to ascertain the functioning capacity of the urinary tract by repeated ultrasound examinations at 20 minutes intervals for 60-120 minutes observation periods[9,10,]. Furthermore, the stimulation by furosemide given to a normal pregnant woman causes the increased production of urine in the fetus and the filled bladder can be demonstrated within 60 minutes[11]. It was reported that intravenous pyelography, performed by injection of contrast material into maternal circulation was useful to examine the fetal kidney function[12]. In the present case, even direct injection of contrast material into fetal circulation produced no pyelogram of the fetal kidney and bladder. Therefore, bilateral renal agenesis of the fetus was confirmed. We also recognized anhydraminos, severe IUGR, breech presentation, scaphoid head, bell-shaped thorax

without breathing movement and a flexed-spine posture with bowed legs and feet.

Results of ultrasound-guided cord blood sampling at 31 weeks indicated severe hypoproteinemia and anemia of which the pathophysiology remains obscure. This malnutrition may be caused by placental dysfunction concluded from low values of unconjugated estriol and hPL. However, the values of electrolytes, BUN, creatinine, and blood gas were normal. It was postulated that the placenta in this case impaired the protein synthesis, whereas, the capacity of exchange or transfer of the compounds like electrolytes or oxygen was preserved.

Consequently, in cases of severe IUGR with oligohydramnios, congenital anomalies of the fetus should be intensively examined by ultrasonography as well as the ultrasound-guided sampling of umbilical cord blood in consideration of the possibility of Potter syndrome.

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