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Studies on α-Fetoprotein and Human Placental Lactogen in Blood of Pregnant Women and Umbilical Cord

(AFP/HPL/pregnant women)

KIYOSHI HASEGAWA[a], FUMINORI MURAO[a], ISAO MATSUNAGA[a], TOSHIHIRO TAGASHIRA[b], NOBUYUKI IBA[a], and MANABU KITAO[a]

[a]*Department of Obstetrics and Gynecology, Shimane Medical University, Izumo 693 and* [b]*Department of Obstetrics and Gynecology, Sakaiminato Saiseikai Hospital, Sakaiminato 684, Japan*

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Blood AFP and HPL of normal pregnant women were detectable at the end of the 3rd month of gestation, increased with the number of months of gestation reached the peak at around the end of the 9th month and leveled off or tended to fall off thereafter ; after delivery, AFP decreased to about half the level at delivery on the 5th puerperal day, while HPL was undetectable two hours after delivery. Umbilical arterial blood AFP showed higher levels than maternal blood AFP, and a good correlation was seen. Umbilical arterial HPL showed lower levels than maternal blood HPL, and here there was no correlation. Blood AFP and HPL of pregnant women showed no diurnal variations. Blood AFP of pregnant women showed high levels in cases of intrauterine fetal death and twin pregnancy, while HPL showed low levels in cases of intrauterine fetal death and high levels in twin pregnancy. Both AFP and HPL were within the normal limits in cases of an anencephalic, Rh(o) negative and diabetic pregnant women. In hydatidiform mole, both AFP and HPL were undetectable.

The discovery of α-fetoprotein (AFP), fetal protein dates back to 1956 when Bergstand and Czar (1) found a component which exists between albumin and α-globulin and which does not exist in adult serum as determined by electrophoresis on human fetal serum.

In 1970, the method of determination, particularly radioimmunoassay (RIA) was perfected and AFP was demonstrated in maternal blood by Purves and Geddes (2). Recently, AFP has attracted attention as a method of predicting fetal distress since it is derived from the fetus and amnion (3).

Meanwhile, human placental lactogen (HPL) has come to be known as simple protein hormone originating from syncitiotrophoblastic cell of the placenta since Higashi in 1961 (4) extracted it from human placenta and reported that it had a lactogenic action.

As it secretes very rapidly and has a short half life in serum, HPL determined periodically is now used as an index for the growth of placental tissue or placental function.

Diagnosis of the fetal environment in the uterus is an important subject in modern obstetrics and recently biochemical studies are being done. Aiming

at clinical application of these two methods of determination, we determined AFP and HPL in normal and abnormal pregnancies.

MATERIALS AND METHODS

Subjects

Blood was collected from 50 normal pregnant Japanese women at each month of pregnancy, 15 cases of abnormal pregnant women and umbilical artery and vein of 20 newborn infants. The blood was centrifuged at 3,000 rpm for five minutes and the supernatant fluid was collected and frozen to -20°C for storage until determinations of AFP and HPL.

Method of Determination

1) AFP was determined using the Kaken AFP-RIA Kit (RCC)
2) HPL was determined using the Kaken HPL-RIA Kit (RCC), Determination of the sample was made in duplicate.

When possible, HPL and AFP were determined simultaneously in the same-sample.

RESULTS

1) Maternal Serum AFP and HPL at Each Month of Normal Pregnancy

According to results of determination of maternal blood AFP in 50 cases, the AFP level was 26.3 ± 3.4 ng/ml at the 4th month of pregnancy, increased in proportion to the number of months of gestation thereafter, peaked to show 163.4 ± 56.0 ng/ml at the 9th month, and reached a plateau or tended to decrease thereafter. The levels were 160.9 ± 46.3 ng/ml at the 10th month of pregnancy and 122.0 ± 32.0 ng/ml immediately after delivery.

At the 5th day of puerperium, levels decreased to 56.2 ± 26.5 ng/ml, to about one half the level at delivery (Fig. 1). According to results of determi-

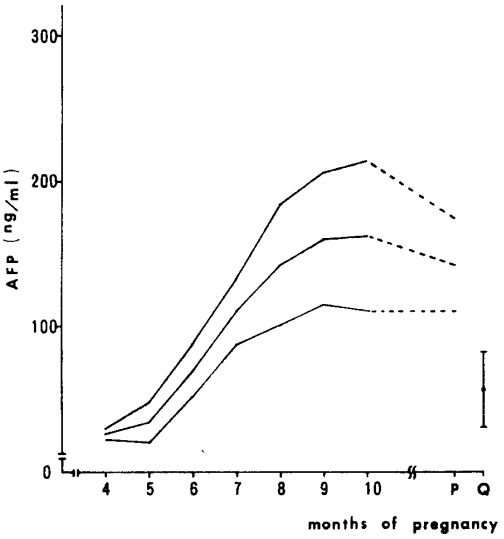

Fig. 1. Changes in maternal serum AFP concentration throughout months of pregnancy. P : 2 hr after delivery, Q : 5th day of puerperium.

nation of maternal blood HPL in 50 cases, the HPL level was 1.2 ± 0.8 μg/ml at the 4th month of pregnancy, increased in proportion to the number of months of gestation thereafter, peaked to show 7.70 ± 3.4 μg/ml at the end of the 9th month, and formed a plateau or tended to decrease thereafter. The level was 7.38 ± 3.1 μg/ml at the 10th month of pregnancy and was undetectable two hours after delivery (Fig. 2).

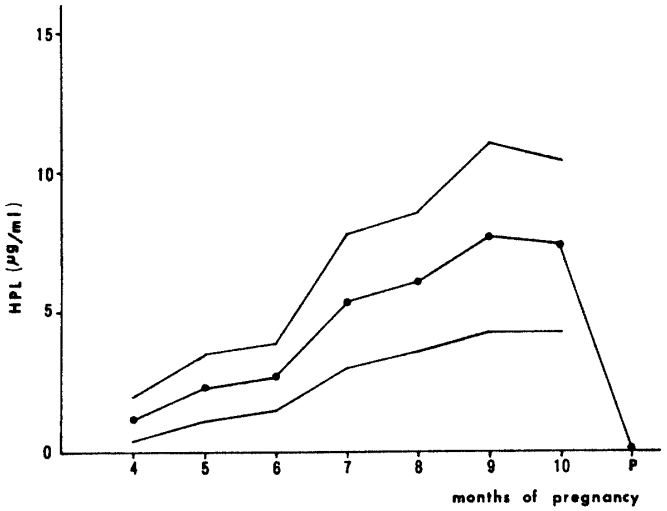

Fig. 2. Changes in maternal serum HPL concentration throughout months of pregnancy. P : 2 hr after delivery.

2) Maternal Blood, Umbilical Arterial, Venous Blood AFP and HPL

In 20 cases, the umbilical arterial and venous blood AFP level was significantly higher than the maternal blood AFP level, with 152.2 ± 46.3 ng/ml for the maternal AFP, 124.3 ± 32.1 μg/ml for the umbilical arterial blood AFP and 112.3 ± 30.2 μg/ml for the umbilical venous AFP. There was a correlation between the maternal blood AFP and the umbilical arterial (r = 0.72) as shown in Table 1.

TABLE I. *Concentration of AFP in Maternal, Umbilical Artery and Umbilical Vein Blood*

Sample	Number	Concentration of AFP (mean±S. D.)
Maternal serum	20	152.2±46.3 ng/ml
Umbilical artery	18	124.3±32.1 μg/ml
Umbilical vein	20	112.3±30.2 μg/ml

In 20 cases, the maternal blood HPL level was 8.5 ± 2.1 μg/ml, the umbilical venous blood HPL was 0.2 ± 0.05 μg/ml and the umbilical arterial blood HPL was undetectable. The maternal blood HPL level was about 40 times higher than the umbilical venous blood HPL level (Table 2).

TABLE II. *Concentration of HPL in Maternal, Umbilical Artery and Umbilical Vein Blood*

Sample	Number	Concentration of HPL (mean±S. D.)
Maternal serum	20	8.5±2.1 μg/ ml
Umbilical artery	20	Undetectable
Umbilical vein	20	0.2±0.05 μg/ml

3) Diurnal Variations

Blood was collected every four hours from two subjects for AFP and from four subjects for HPL at 40 weeks' gestation, and AFP and HPL were determined. AFP showed no diurnal variations in the two cases (Fig. 3). HPL reached the highest level at 11 a. m. but did not show any remarkable diurnal variations in all four cases (Fig. 4)

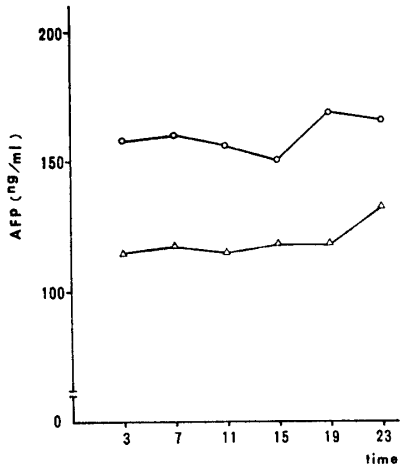

Fig. 3. Diurnal variations in serum AFP concentration in two cases of 40 weeks' gestation.

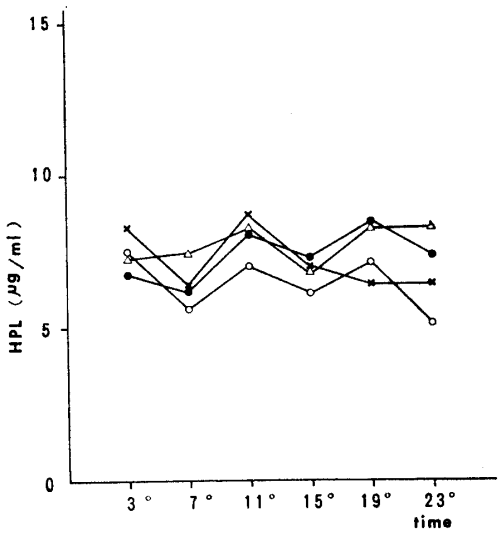

Fig. 4. Diurnal variations in serum HPL concentration in four cases of 40 weeks' gestation.

4) AFP and HPL in Abnormal Pregnancy

(1) Hydatidiform mole

Both AFP and HPL were undetectable in all of the three cases of hydatidiform mole (Figs. 5, 6).

(2) Abortion and Intrauterine Fetal Death

In two with an intrauterine fetal death who were admitted for threatened abortion, a test done two weeks earlier showed AFP to be high in both cases, HPL to be normal in one case and low in one case, with AFP 300 ng/ml, 342 ng/ml and HPL 5.5 μg/ml, 3.0 μg/ml.

One patient with high AFP levels and normal HPL levels had a fetus with a

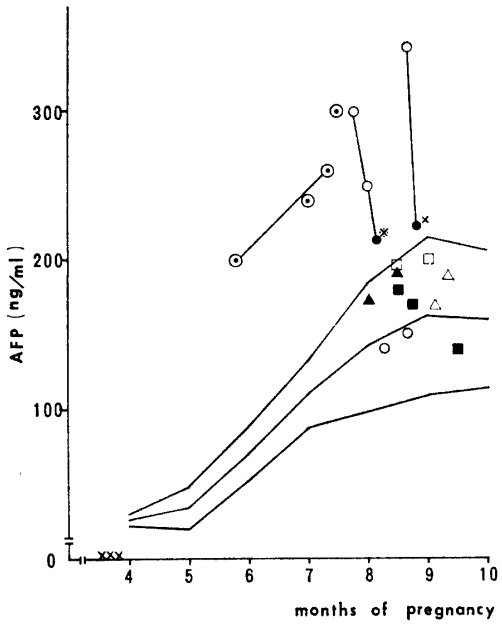

Fig. 5. Maternal serum AFP levels in each month of normal pregnancy and specific cases. ×hydatidiform mole, ◉ twin, ▲ anencephalus, □ diabetus, ■ toxemia, △ isoimmunization, ○ threatened abortion and premature labor, ●※ 3 days after intrauterine death, ●× 2 days after intrauterine death.

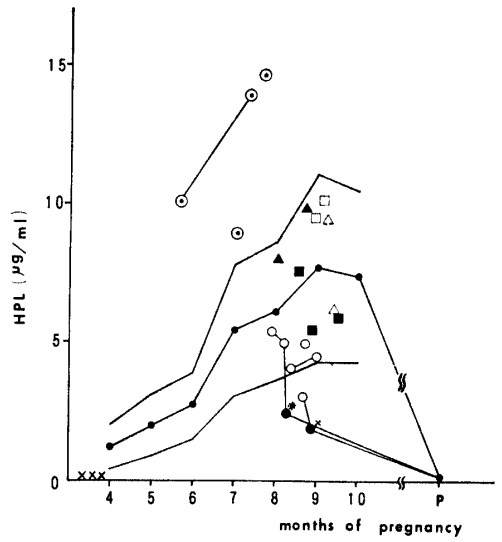

Fig. 6. Maternal serum HPL levels in each month of normal pregnancy and specific cases. × hydatidiform mole, ◉ twin, ▲ anencephalus □ diabetus, ■ toxemia, △ isoimmunization ○ threatened abortion and premature labor, ●※ 3 days after intrauterine death, ●× 2 days after intrauterine death, P : 2 hr after delivery.

high degree of external deformity.

One patient with high AFP levels and low HPL levels had a small for date baby.

In two with premature labor, the AFP level was within normal limits, while

the HPL level was a lower limit of the normal level and tended to decrease (Figs. 5, 6).

At the third day after presumed intrauterine fetal death, the AFP level was 212 ng/ml and the HPL level was 2.5 μg/ml. In another, the AFP level was 310 ng/ml and HPL 2.0 μg/ml (Figs. 5, 6).

(3) Late Toxemic Pregnancy

In three mild cases, both AFP and HPL levels were within the normal limits. In one severe case of intrauterine fetal death, the AFP level was high and the HPL low (Figs. 5, 6).

(4) Twin Pregnancy

In three cases of twin pregnancy, both maternal blood AFP and HPL showed high levels in all cases, the AFP being 200 ng/ml, 260 ng/ml and 300 ng/ml and HPL 10.2 μg/ml, 14.4 μg/ml and 14.8 μg/ml (Figs. 5, 6).

(5) Anencephalus

In two cases of anencephalus in the 9th month of pregnancy, the maternal blood AFP level was at the upper limit of normal levels and the HPL level was within normal limits, with AFP 172 ng/ml, 190 ng/ml and HPL 7 μg/ml, 6 μg/ml (Figs. 5, 6).

(6) Rho (−) Pregnant Women

All patients showed no Rh(o) (+) antibody. Maternal blood AFP and HPL levels were both within normal limits (Figs. 5, 6).

(7) Diabetic Pregnant Women

In two cases of class B (White B classification), the maternal blood AFP level was 196 ng/ml at 34 weeks' gestation and 200 ng/ml at 36 weeks' gestation, showing the upper limit of normal levels, while the HPL level likewise showed the upper limit of normal levels at 9.6 μg/ml, 10.5 μg/ml (Figs. 5, 6).

DISCUSSION

1. Maternal Blood AFP and HPL in Normal Pregnancy

Serum AFP and HPL of normal pregnant women increased gradually with the advance of pregnancy, reached a peak at around the end of the 9th month of pregnancy and then formed a plateau, or tended to decrease thereafter as illustrated in Figs. 1, 2.

These results are in agreement with the reports of Seppälä and Ruoslahti (5), Tagashira (6), Iwasa (7).

After delivery, AFP decreased but did not metabolize and disappeared quickly in the maternal blood as did HPL but declined slowly, day by day.

Tagashira (6) estimated the half life to be three days. When maternal blood AFP and umbilical arterial-venous blood AFP during labor were examined, the AFP level in the umbilical arterial blood was higher than that in maternal blood and there was a correlation between the two. AFP disappeared from the maternal blood after delivery. In the case of hydatidiform mole, AFP was not demonstrated in the maternal blood.

From the findings above, maternal blood AFP is considered to be transferred from the fetus and can possibly be used as an index for fetal conditions.

Maternal blood HPL after delivery decreased rapidly after expulsion of the placenta and was undetectable 2 hr later. The half life is short, and according to Teoh *et al.*(8), it was about 20 min.

In the maternal blood, before labor, estimated on the 3rd day of intrauterine fetal death, however, the HPL level was low but still detectable at 2 μg/ml.

When maternal blood HPL and umbilical arterial venous blood HPL at labor were examined, the HPL level in umbilical arterial and venous blood was extremely low or undetectable compared with that in maternal blood and there was no correlation between the two.

In light of the results and a report (8) that HPL is produced only in the placenta, it is suggested that changes in maternal serum HPL are a reflection of placental changes on maternal blood, and HPL may be a significant index for evaluating placental function.

As to the diurnal variations in blood HPL of pregnant women, not much change was observed. Similar findings are given in the reports of Mochizuki (9) and Teoh *et al.*(8).

Kumasaka (10) found that the HPL level is relatively low in the a. m. with transient high levels around noon and that collecting blood always at a fixed time is necessary when measuring HPL.

2. AFP and HPL in Abnormal Pregnancy

Nothing definite can be said about abnormal pregnancy since the number of cases was small, but we obstained some interesting findings.

Blood HPL and AFP levels in hydatidiform mole were all below the limits of measurement. Regarding AFP in hydatidiform mole, a similar finding is seen in the report of Tagashira (6).

As to HPL, Mochizuki reported that the HPL level was low or below the limits of measurement (11).

In normal pregnancy, AFP and HPL are detectable in the latter half of the 3rd month of pregnancy.

Accordingly, AFP and HPL are useful as an auxiliary method of diagnosis in distingushing hydatidiform mole from normal pregnancy.

In cases of intrauterine fetal death, abnormally high levels of AFP were observed prior to the fetal death in two cases with long-term pre-care.

Tagashira (6) likewise reported that maternal blood AFP showed abnormally high levels at the time of intrauterine fetal death due probably to an increase in AFP production by the dead fetus and accentuation of the placental permeability.

Seppälä and Ruoslahti (3) reported that AFP showed high levels, 530 ng/ml or over in 60% of cases involving fetal distress.

As to the maternal blood HPL in two who had been followed since before fetal death, levels were much lower than those in the corresponding period of normal pregnancy in one case and a lower limit of normal levels in another.

The one which showed a level equivalent to the lower limit of normal levels had a high degree of external deformity but no particular abnormality was seen in the placenta.

Here the maternal blood HPL level on the second day of presumed death was 2.5 μg/ml and was undetectable 2 hr after delivery.

According to Yaoi *et al.*(12), HPL is secreted from the placenta itself and unlike E_3, is not under control by the fetus, and at the terminal stage of pregnancy, the placenta itself has a considerable reserve capacity and it is only when a change in the placenta occurs extensively and simultaneously that a fall in HPL takes place.

Accordingly, it is possible that results of blood HPL are not constant even in the case of abnormal pregnancy at the terminal stage of pregnancy, they reported.

Letchworth and Chard (13) reported that the probability of fetal distress or asphyxia of newborn infants was 71% when the blood HPL level was 4 μg/m or below three consecutive times in cases of continual determination after 35 weeks' gestation.

Teoh *et al.*(8) reported that the danger zone for the fetus after 30 weeks' gestation was 4 μg/ml or below.

Therefore, the simultaneous determination of maternal blood AFP and blood HPL is of significance as a means of diagnosing latent fetal distress and determining the fate of the fetus. Particularly, in the case of high AFP and low HPL levels, careful management of the fetus is required.

According to Tagashira (6), blood AFP in pregnant women with a toxemia did not show a fixed trend in mild cases but high levels were seen in 3 out of 4 severe cases. Yagi (14) stated that maternal blood AFP rather showed low levels in serious cases, while Seppälä (15) reported that there was no correlation between the intensive degree of toxemic pregnancy and maternal blood AFP.

According to our results, the maternal blood AFP was within the normal limits in mild cases and showed a high level in one serious case, and such resulted in intrauterine fetal death at 33 weeks.

Blood HPL in pregnant women with a toxemia was within normal levels in mild cases and there were no characteristic findings.

In cases of fetal death, blood HPL showed a low level, 3 μg/ml.

Kaneko *et al.*(16) maintained that the severity of toxemic pregnancy did not parallel the HPL levels. Genazzani *et al.*(17) reported that the HPL level was lower in preeclamptic pregnancy than in normal pregnancy, very low in the case of a premature delivery and low particularly in cases of placental dystrophy or infarct.

Teoh *et al.*(8) determining blood HPL in 230 pregnant women with toxemia reported that the level was less than 4 μg/ml in 54 such women after 30 weeks' gestation, 13 had stillbirth and that severe cases were more liable to enter the danger zone than mild ones.

When, therefore, AFP is high and HPL is low in cases of a toxemia, the

fate of the fetus is probably poor.

The maternal serum AFP level in cases of anencephalus at the 9th and 8th month of pregnancy was within the normal limits as was also reported by others (18, 19).

Accordingly, maternal blood AFP is of little significance in the diagnosis of anencephalus.

However, Tagashira (6) maintains that determination of amnion AFP is useful since the maternal blood AFP level is within the normal limits while amnion AFP shows high levels in pregnant women with anencephalus as compared with cases of a normal pregnancy.

Likewise the blood HPL level in pregnant women with anencephalus was within the normal limits. It appears that the placental function is more or less normal in cases of anencephalic pregnancy.

Maternal blood AFP in twin pregnancy showed higher levels than in normal pregnancy.

Tagashira (6) found that AFP showed high levels even in a stage where it was difficult to diagnose twin pregnancy by roentgenography and suggested the possibility of an auxiliary diagnosis by determination of AFP.

Maternal blood HPL as well as AFP in twin pregnancy showed higher levels than in cases of normal pregnancy.

Maternal serum AFP in two cases of diabetic pregnant women (White classification Class B) showed higher levels compared with cases of normal pregnancy.

Norgaard-Pedersen and Klebe (20) stated that AFP was almost within the normal limits in Class A, showed high levels in Class B — F, was almost normal in mild cases and showed high levels in serious cases.

Accordingly, maternal blood AFP may be useful as a means of determining the severity of diabetic pregnancy.

Blood HPL of two pregnant women belonging to Class B diabetic pregnancy was within the limits found in the corresponding period of normal pregnancy.

Hashimoto (21) reported that HPL levels were sometimes low in the case of diabetes mellitus.

Genazzani *et al.*(17) held that the HPL levels were rather high in mild cases.

Crosignani *et al.*(22) reported that HPL was normal in the case of treatment with sufficient doses of insulin but high in case of inadequate treatment.

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