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Protein Patterns of Blood Plasma in Pregnant Women by SDS/polyacrylamide Gel Electrophoresis

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SDS/polyacrylamide Gel 電氣泳動에 依한 姙娠한 女子 血漿蛋白質의 패턴

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요 약

非姙娠 女子, 姙娠된 女子 및 正常인 男子의 血漿蛋白質을 SDS/polyacrylamide 젤 電氣泳動으로 分析하였다.

分子量 10,000에서 110,000 dalton 사이의 正常인 男子와 非姙娠 女子의 血漿蛋白質의 패턴은 同一하였다. 本 硏究에서 非姙娠 女子와 姙娠된 女子의 血漿蛋白質을 比較하였을 때 새로운 band들이 나타나지는 않았지만 어떤 蛋白質 band들은 姙娠의 初期, 中期 및 末期에 따라서 量的으로 增加 또는 減少되었다.

各 band들의 分子量을 測定한 結果 分子量 76,000 dalton 以上의 蛋白質들이 姙娠中 增加 또는 減少되어 짐을 알 수 있었다. 즉 分子量 86,000 dalton의 蛋白質은 姙娠 中期 까지는 增加되지 않았으나 姙娠 末期에는 增加되었다. 分子量 91,000~105,000 dalton 사이의 蛋白質들은 姙娠의 期間이 길어짐에 따라 대체로 量이 增加되었다.

反面에 分子量 94,000 dalton의 蛋白質은 姙娠 中期 까지는 量이 若干 減少되었으나 末期에는 오히려 增加되었다. 그리고 分子量 99,000 dalton의 蛋白質은 姙娠 初期 까지는 變化가 없었으나 中期에서 末期로 감에 따라 漸次的으로 增加되었다.

그러므로 우리들은 姙娠된 女子 血漿의 蛋白質 패턴을 調査함으로써 姙娠의 期間을 알 수 있음을 提示하는 바이다.

INTRODUCTION

During pregnant period, metabolic rate is usually elevated when compared to non-

pregnant women, because certain constituents of plasma are increased in some cases and decreased in other cases.

Smithies (1959), who classified the first, the second, and the third trimester of pregnancy, found "pregnancy zone" from the women in the third trimester of pregnancy in starch gel electrophoresis. He further observed that the protein in the "pregnancy zone" was not combined with hemoglobin. Later, Padreira *et al.* (1962) reported that migration of the "pregnancy band" in the electrophoresis was faster than that of plasma α_2-globulin in starch gel elctrophoresis.

Choi and Ha (1985) compared protein patterns in maternal plasma of women who delivered a female-child to those of women delivered a male-child using SDS/polyacrylamide gel electrophoresis.

This study deals with the protein patterns in plasma of non-pregnant women and pregnant women ranging 10,000~110,000 daltons in molecular weights by using SDS/polyacrylamide gel electrophoresis. In a good agreement with Smithies' report (1959), the "pregnancy zone" was observed in starch gel electrophoresis; however, it was not the case when the starch gel was substituted by the SDS/polyacrylamide gel in the electrophoresis.

MATERIALS AND METHODS

Blood was obtained from healthy men, women who have not experienced pregnancy, and pregnant women. The blood was centrifuged at 4°C for 20 min at 10,000×g (Hitachi 20 PR-5). Then one volume of the supernatant from this centrifugation tube was added to the 19 volume 0.0625M Tris/HCl buffer (pH 6.8) containing 2% SDS (Sodium Dodecyl Sulfate, Aldrich Chemical Co.), 5% 2-mercaptoethanol (Junsei Chemical Co.), and 10% sucrose (Sigma). The mixture was maintained at 100°C for 90 sec, chilled, and used as sample for electrophoresis with addition of a small amount of bromophenol blue (Junsei Chemical Co.).

For the determination of molecular weights of protein bands, lysozyme (mol. wt. 14,300), β-lactoglobulin (mol. wt. 18,400), trypsinogen (mol. wt. 24,000), egg albumin (mol. wt. 45,000), and bovine albumin (mol. wt. 66,000) (Sigma) was used. The molecular weight of each band was calculated by using the procedures described by Banker and Cotman (1972), Neville (1971), Peacock and Dingman (1968), Rodbard and Chrambach (1971), and Swank and Munkres (1971).

The gel and buffer systems in SDS/polyacrylamide gel electrophoresis were based on the methods of Laemmli (1970) and Neville (1971).

Electrophoresis was carried out at 5°C at 100V for 8 hours by using the vertical slab gel system (Electrophoresis system of LKB). After the electrophoresis, the gel was stained with Coomassie Brilliant Blue R 250 (Sigma) at 37°C for 2 hours, and destained with isopropanol and acetic acid solution. These destained gels were scanned with a

DMU-330 densitometer (Toyo) at 570 nm.

RESULTS

The protein patterns of plasma from normal male individuals, ranging 10,000~110,000 daltons in molecular weights, were separated by SDS/polyacrylamide gel electrophoresis, and are shown in Fig. 1. Molecular weights of representative 12 bands are shown in Table 1. In protein patterns of normal men, non-pregnant women, and pregnant women, the highest intensity of band stained with Coomassie Brilliant Blue R 250 was band d of 64,000 dalton in molecular weight (Figs. 1~5). The high molecular weight portion ranging 10,000~110,000 daltons showed relatively complicated patterns when compared with low molecular weight portion.

When the protein patterns of normal male individuals are compared with those of non-pregnant women, no significant difference in protein patterns was observed and the densities of each band appeared to be similar to each other (Figs. 1, 2).

When the protein patterns of non-pregnant women (Fig. 2) were compared with those of pregnant women (Figs. 3~5), there was no significant difference in protein patterns but the quantities of proteins were significantly different.

In the plasma protein patterns of pregnant women, band d (mol. wt. 64,000 dalton) was decreased significantly with advancing gestation. The increase and decrease in quantity of protein ranging 76,000~105,000 daltons in molecular weights also observed

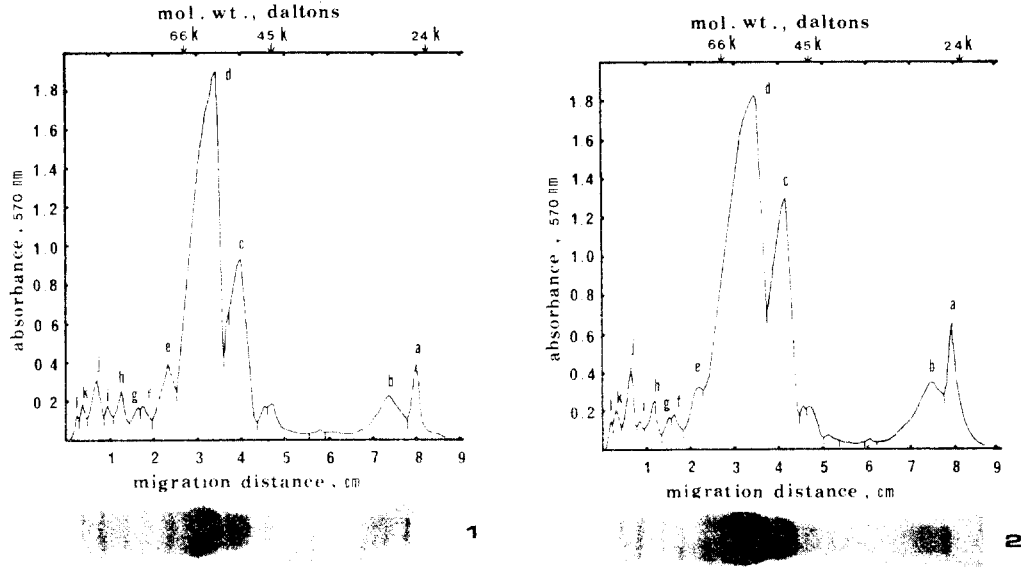
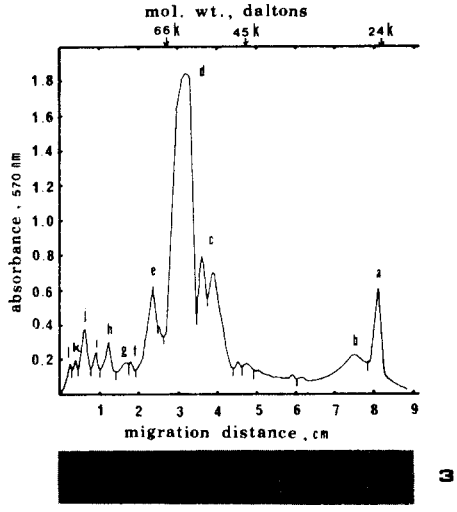
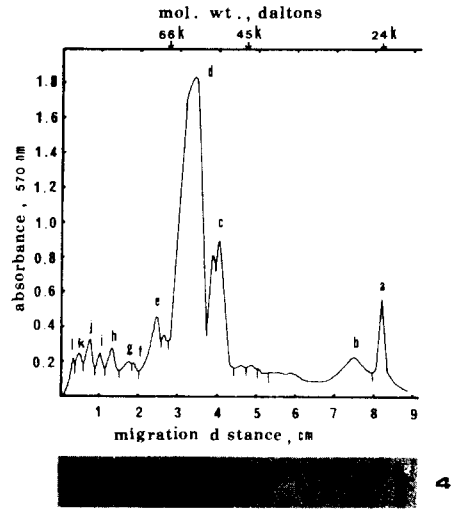


Fig. 1. Protein patterns of blood plasma in normal male.
Fig. 2. Protein patterns of blood plasma in normal female.





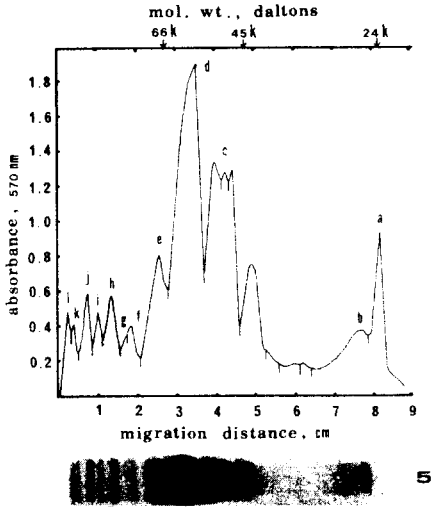


Fig. 3. Protein patterns of blood plasma of pregnant woman in the first trimester of pregnancy.

Fig. 4. Protein patterns of blood plasma of pregnant woman in the second trimester of pregnancy.

Fig. 5. Protein patterns of blood plasma of prepnant woman in the third trimester of pregnancy.

to be remarkable. That is, bands f and g (mol. wt. 76,000 and 80,000 daltons, respectively) were not increased in their quantity in the second trimester of pregnancy (Fig. 4), but were increased in the third trimester of pregnancy (Fig. 5). The bands h and i (mol. wt. 86,000 and 91,000 daltons, respectively) showed gradually higher intensity as they were progressing from the first trimester of pregnancy (Fig. 3) to the third trimester of pregnancy.

Meanwhile, bands j and k (mol. wt. 94,000 and 99,000 daltons, respectively) were

Table 1. Molecular weights of blood plasma of non-pregnant women.

bands	mol. wt. (daltons)	bands	mol. wt. (daltons)
a	24,000	*g	80,000
b	28,000	*h	86,000
c	51,000	*i	91,000
d	64,000	*j	94,000
e	70,000	*k	99,000
*f	76,000	*l	105,000

Asterisks (*) indicate bands which changes remarkable in quantity during pregnancy.

slightly decreased in their quantity in the second trimester of pregnancy, but were increased significantly in the third trimester of pregnancy. Band 1 (mol. wt. 105,000 dalton), which showed relatively small amounts in non-pregnant women, was increased at a higher rate in quantity than other bands as progressing to gestation.

DISCUSSION

Many authors reported the increase of hormones, synthesized in placenta, in the blood of fetus and in the maternal serum during pregnancy: human chorionic gonadotropin (Boroditsky *et al.*, 1975) and progesterone (Tulchinsky and Okada, 1975). Hormones such as gonadotropin-releasing hormone (Siler-Khodr *et al.*, 1984), β-endorphin, β-lipotropin, and adrenocorticotropic hormone (Genazzani *et al.*, 1984), and pregnancy-specific β_1-glycoprotein (Haddow *et al.*, 1984; Sϕrensen, 1978) were also reported to be significantly elevated during pregnancy.

Enzymes such as plasma alkaline phosphatase (Adeniyi and Olatunbosun, 1984), glucose dehydrogenase, β-glucoronidase and N-acetyl-β-glucosaminidase Huddleston *et al.*, 1971), formed in placenta, were transferred into maternal plasma. In addition, among the products of fetus, which can enter into maternal plasma, alpha-fetoprotein has been widely studied. For example, a large amount of alpha-fetoprotein was found in the amniotic fluid when the fetus had a neural tube defects, but a part of it was transferred to maternal plasma (Adams *et al.*, 1984; Garoff and Seppälä, 1975; Milunsky *et al.*, 1982). Maroulis *et al.* (1971) described that fetal cells and globulin can cross into the maternal circulation during pregnancy, and stimulate the production of antibodies to fetal red and white cells, platelets, and IgG isoantigens.

As mentioned above, the components of plasma in pregnant women were increased on account of the produced substances in fetus, and the active maternal metabolism itself. Thus, blood plasma contains lots of protein components including enzymes, hormones, and other proteins.

Smithies (1959) observed "pregnancy zone" in the serum of pregnant women using

starch gel electrophoresis, which occurred in the third trimester of pregnancy or the recently delivered. But he did not describe of what substances "pregnancy zone" was composed. Pedreira *et al.* (1972) observed that "pregnancy band", which corresponds to the "pregnancy zone" of Smithies (1959), had migrated between α_2-globulin and β-globulin in starch gel electrophoresis. The proteins separated by starch gel electrophoresis are very large macromolecules which are composed of native protein. But in this study, the specific "pregnancy band" was not detected by SDS/polyacrylamide gel electrophoresis. Thus, to determine the molecular weight of the protein of "pregnancy band", the protein of the band which was separated by starch gel electrophoresis is eluted, and the eluted protein must be electrophoresed by SDS/polyacrylamide gel electrophoresis. This study investigated about the changes in quantity of the whole protein patterns of plasma in pregnant women and about the determination of the molecular weights of these proteins, since the maternal metabolism showed higher activity in the whole body of pregnant women than non-pregnant women.

It appeared that the quantity of protein in bands ranging 76,000~105,000 daltons in molecular weights was increased with advancing gestation. In pregnant women, band f (mol. wt. 76,000 dalton), which increased gradually as it advances from the first trimester of pregnancy to the third trimester of pregnancy, might be transferrin. Since the function of this protein is transporting irons it should role in certain metabolism which is indispensable to the fetus. In non-pregnant women, the amount of protein of band 1 (mol, wt. 105,000 dalton) was small, but there was such phenomenon that the increasing ratio of band 1 protein was twice as much as that of any other proteins in pregnant women. It seems that this protein is one of the denaturated protein components in "pregnancy band", which was reported by Smithies (1959) and Pedreira *et al.* (1962). It was observed that albumin was remarkably decreaed with advancing gestation. This phenomenon may have some relation with hemodilution (Pedreira *et al.*, 1962).

We suggest that the diagnosis of pregnancy in the early trimester and the elapsed time of pregnancy could be detected by investigations of the changing quantity of plasma protein of pregnant women. And the possibility to detect a pathological malformation of fetus can also be interpreted on the basis of protein patterns in the serum of pregnant women.

ABSTRACT

The plasma protein patterns of non-pregnant women, pregnant women, and normal male individuals were analyzed by SDS/polyacrylamide gel electrophresis.

When the protein patterns of plasma of normal male individuals ranging from 10,000 to 110,000 daltons in molecular weights are compared to non-pregnant women, their protein patterns were the same. In this study, when the plasma of non-pregnant women are compared to pregnant women, no bands were occurred newly, but the quantity of some protein

bands were increased or decreased during the pregnant periods.

According to the results of measuring the molecular weights of the characteristic protein patterns, which are increasing or decreasing during the pregnancy as compared to the non-pregnant women, it was observed that the proteins over 76,000 daltons in molecular weights were concerned in the facts mentioned above. That is, the protein of 86,000 dalton in molecular weight was not increased in quantity until the second trimester of pregnancy, but was increased in the third trimester of pregnancy. The proteins of 91,000~105,000 daltons in molecular weights were gradually increased in accordance with the periods of pregnancy.

On the contrary, the protein of 94,000 dalton was rather decreased by the second trimester of pregnancy, but increased in the third trimester of pregancy. And the band of 99,000 dalton was not changed in quantity significantly until the first trimester of pregnancy, but increased continuously from the second trimester of pregnancy to the third trimester of pregnancy.

We tentatively suggest that the stages (the first, the second, and the third trimester) of pregnancy can be identified by the study on the protein patterns of the specific bands in the blood plasma of pregnant women.

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