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## Abstract

To study changes in hemorheologic properties during pregnancy, erythrocyte deformability was measured by an electron spin resonance (ESR) method. The results obtained by this method showed that erythrocyte deformability in normal pregnancy decreased significantly in the first trimester compared with nonpregnant controls, and continued to decrease slightly as pregnancy progressed. On the other hand, erythrocyte deformability in severe pregnancy-induced hypertension (PIH) was significantly lower than that in the third trimester of normal pregnancy. Additionally, we found that the hematocrit level needed for erythrocytes to exhibit high deformability is lower during pregnancy. These results suggest that hemodilution in normal pregnancy, so-called hydremia, compensates for the decrease in erythrocyte deformability. Conversely, since erythrocytes become less deformable in a hemoconcentration condition in severe PIH, microcirculatory disturbance of various organs, including the uteroplacental unit, may occur. The lowered erythrocyte deformability may be one of the important pathologic features in PIH.

**KEYWORDS:** erythrocyte deformability, electron spin resonance, pregnancy-induced hypertension, hydremia

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## Changes in Erythrocyte Deformability in Normal Pregnancy and Pregnancy-Induced Hypertension, as Revealed by Electron Spin Resonance

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To study changes in hemorheologic properties during pregnancy, erythrocyte deformability was measured by an electron spin resonance (ESR) method. The results obtained by this method showed that erythrocyte deformability in normal pregnancy decreased significantly in the first trimester compared with nonpregnant controls, and continued to decrease slightly as pregnancy progressed. On the other hand, erythrocyte deformability in severe pregnancy-induced hypertension (PIH) was significantly lower than that in the third trimester of normal pregnancy. Additionally, we found that the hematocrit level needed for erythrocytes to exhibit high deformability is lower during pregnancy. These results suggest that hemodilution in normal pregnancy, so-called hydremia, compensates for the decrease in erythrocyte deformability. Conversely, since erythrocytes become less deformable in a hemoconcentration condition in severe PIH, microcirculatory disturbance of various organs, including the uteroplacental unit, may occur. The lowered erythrocyte deformability may be one of the important pathologic features in PIH.

**Key words:** erythrocyte deformability, electron spin resonance, pregnancy-induced hypertension, hydremia.

Recently, the relationship between hemorheologic properties has been controversial with regards to microcirculatory disturbance, which was thought to be important in the pathogenesis of pregnancy-induced hypertension (PIH). In fact, some hemorheologic properties were reported to change during both normal pregnancy and PIH, such as whole blood viscosity, plasma viscos-

ity, hematocrit *etc.* (1). Erythrocyte deformability has also been thought to be an important factor. Filterability was generally used as an index of erythrocyte deformability (2). However, some shortcomings of this method were noted, including a lack of reproducibility and other factors affecting measurement values (3). Additionally, erythrocyte deformability was considered to be influenced by the suspension conditions, which included intercellular interaction of erythrocytes (hematocrit) and extracellular viscosity (plasma viscosity) (4). Thus, in our present study, the erythrocyte deformability was measured by an electron spin resonance (ESR) method developed by Noji *et al.*, which showed alterations in the form of red blood cells in terms of their average physical behavior (5). The main advantages of this method were high sensitivity, the ability to measure deformability at a high hematocrit concentration, and ease of measuring. The purpose of this study was to elucidate the changes of erythrocyte deformability in both normal pregnancy and PIH by using the ESR method, and to hemorheologically investigate the pathophysiologic conditions in PIH.

### Patients and Methods

Ten nonpregnant women, 30 pregnant women (first, second and third trimesters, 10 cases each), 10 7th puerperal day cases and 7 severe PIH cases in the third trimester were included in the study.

Freshly drawn heparinized venous blood was centrifuged at 3,000 rpm for 10 min at 4°C, using a swing bucket rotor. The plasma and buffy coat were removed by an aspirator. The packed cells were then washed three times with Hanks' buffer solution (pH: 7.4, Nissui Co., Tokyo, Japan). The washed, packed cells were suspend-

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ed in hematocrit 35 % in a Hanks' buffer solution containing 8 % dextran 40 (Sigma Co., St. Louis, MO, USA). The suspension was incubated for 10 min at 37°C in a polypropylene tube coated on the inside with a thin film of fatty acid spin label (5-doxy stearic acid, Sigma Co.). The final spin label concentration in the suspension was kept at approximately 65  $\mu\text{mol/l}$ .

ESR spectra were obtained at room temperature (23–25°C) on the ESR spectrometer (a model JES-FE1XG, JEOL, Tokyo, Japan) with 100 KHz field modulation (modulation width = 4G). The microwave power (approximately 9.4GHz) was kept at 30 mW, at which no power saturation effect was observed. The labeled cell suspension (ca. 6 ml) was circulated through a flat quartz ESR sample cell (gap: 0.27mm) by using a peristaltic pump (model AC-2120, Atto Co., Tokyo, Japan). The ESR cell was placed in the cavity resonator with the flat surface held perpendicular to the external magnetic field. The magnitude of the magnetic field was kept at 3,300–3,400 G. The ESR measurement procedure was performed within approximately 2h after incubation.

The coefficient of variation averaged approximately 3 % for intra-assay error. Unless otherwise stated, values were presented as the mean  $\pm$  SD. Statistical analysis was performed by Student's *t*-test with significance taken at  $p < 0.05$ .

## Results

In the top of Fig. 1 are shown ESR spectra of a spin labeled erythrocyte suspension in the absence of flow (dotted line) and under the flow condition (solid line, flow rate: 10 ml/min). The long chain fatty acid spin labels were incorporated into the outside of the lipid bilayer of the erythrocyte membrane, in which the labels were oriented with the chain axes approximately perpendicular to the membrane surface. The nitroxide group of the label, which serves as the source of the electron spin resonance spectrum, has its unique magnetic axis oriented parallel to the chain direction. In the absence of flow, the cells in suspension and the magnetic axes of spin labels are randomly distributed in spatial orientation. Whereas under the shear stress due to flow, the cells partially orient, resulting in a nonuniform spatial orientation of the labels. Consequently, the electron spin resonance spectra under the two conditions exhibit some difference in shape. To characterize the spectral change, the spectrum in the absence of flow was subtracted from the one taken under

the flow condition, as shown in the bottom of Fig. 1. The relative spectral difference, defined as  $\Delta h/h$ , was used as the index of erythrocyte deformability, where  $\Delta h$  is the maximum height of the spectral change and  $h$  is the peak-to-trough amplitude of the spectrum in the absence of flow.

To show the dependence of erythrocyte deformability on shear stress,  $\Delta h/h$  was plotted against the flow rate (Fig. 2). The  $\Delta h/h$  value was increased steeply with the flow rate, reaching a plateau at about 6 ml/min. This dependence of erythrocyte deformability on flow rate was easily reproducible. At the maximum  $\Delta h/h$  (flow rate: 10 ml/min), erythrocyte deformability in the normal third trimester of pregnancy ( $0.324 \pm 0.014$ ) was found to decrease significantly ( $p < 0.001$ ) compared with non-pregnancy ( $0.365 \pm 0.018$ ).

Table 1 shows the changes in erythrocyte deformability in the courses of normal pregnancy and severe PIH. The erythrocyte deformability in normal pregnancy

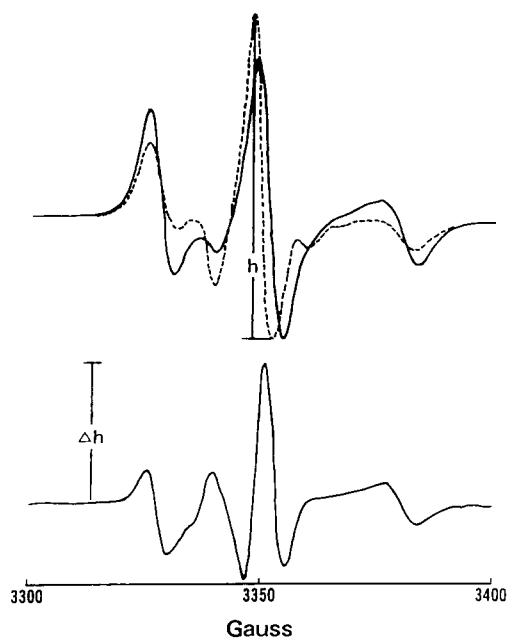


Fig. 1 Electron spin resonance spectra. In the top of the figure, dotted line shows spectrum in the still state of spin labeled erythrocytes suspension. Under the flowing condition (flow rate: 10 ml/min), spectrum changes are shown by the solid line. The bottom of the figure shows difference in spectra calculated by subtracting the dotted line from the solid line. The relative spectral difference defined by  $\Delta h/h$  was used as the index of erythrocyte deformability.

decreased significantly in the first trimester compared with nonpregnancy, and continued to decrease slightly as pregnancy progressed. Erythrocyte deformability in puerperium was nearly equal to nonpregnant controls; erythrocyte deformability in severe PIH decreased even more significantly in comparison with that in the normal third trimester.

As the conditions for erythrocyte-floating suspension were fixed, the results of the present study were thought to be due to changes in the erythrocyte itself. It was thought that extracellular viscosity (plasma viscosity) and intercellular interaction (hematocrit) were also important factors that influenced erythrocyte deformability (4). In fact, it has been reported that hematocrit decreased in normal pregnancy, whereas the decrease of hematocrit tended to be disturbed in PIH (6), and plasma viscosity was thought to increase due to changes in the level of fibrinogen (1). Therefore, the effect of these changes on erythrocyte deformability was examined *in vitro*.

Fig. 3 shows the dependence of erythrocyte deformability on hematocrit and extracellular viscosity. Using the same sample collected from a normal pregnant woman in the third trimester, erythrocyte deformability was measured after changing the hematocrit and dextran concentrations in a suspended medium. In a nonpregnant control, the same procedure was performed. The curves describe two phases: erythrocyte deformability increased with hematocrit to a peak and then declined. Increasing the dextran concentration increased deformability and caused the peak to occur at a lower hematocrit. These characteristics were found to be common in both normal

pregnancy and nonpregnancy. Therefore, erythrocyte deformability was demonstrated to have been influenced

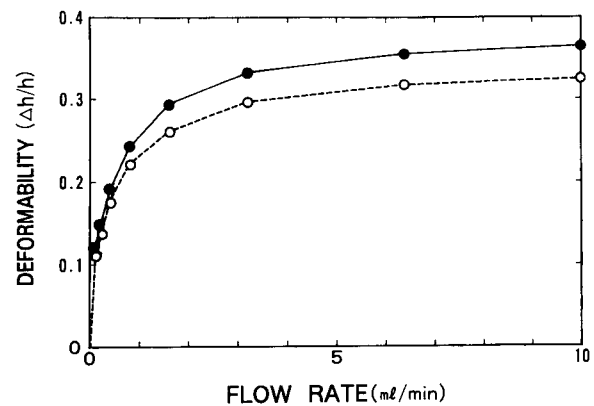


Fig. 2 Dependencies of erythrocyte deformability on the flow rate. The closed circles indicate mean values of nonpregnant controls ( $n = 10$ ), whereas the open circles indicate mean values of normal pregnant women in the third trimester ( $n = 10$ ).

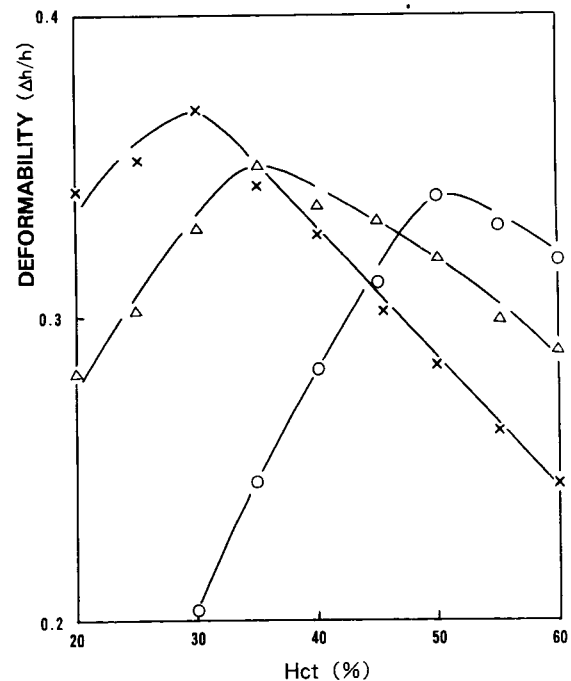


Fig. 3 Dependencies of erythrocyte deformability ( $\Delta h/h$ , flow rate: 10 ml/min) on hematocrit (Hct) and extracellular viscosity in pregnancy. The viscosity of the suspending medium was increased by increasing the dextran concentration. (12%:  $\times$ ), (8%:  $\Delta$ ), (4%:  $\circ$ ).

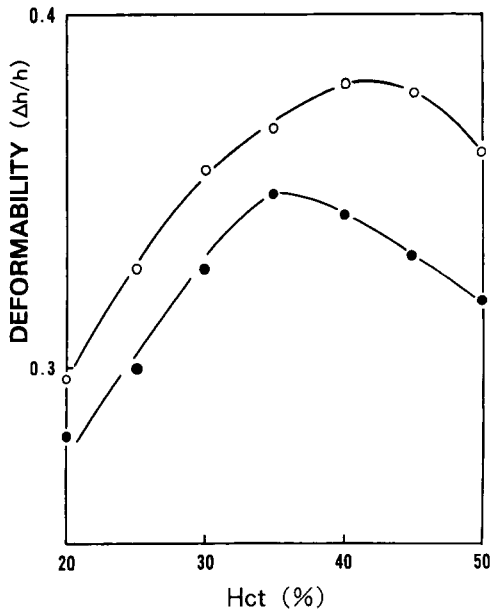
Table 1 Changes of erythrocyte deformability in nonpregnancy, normal pregnancy, puerperium and pregnancy-induced hypertension (severe cases: third trimester)

Group	(n)	Erythrocyte deformability ( $\Delta h/h$ , flow rate: 10 ml/min)
Nonpregnancy	(10)	$0.365 \pm 0.018$
Normal pregnancy	(30)	
First trimester	(10)	$0.329 \pm 0.018^*$
Second trimester	(10)	$0.327 \pm 0.012^*$
Third trimester	(10)	$0.324 \pm 0.014^*$
Puerperium	(10)	$0.360 \pm 0.004$
PIH (severe cases)	(7)	$0.306 \pm 0.014^{**}$

The values are presented as the mean  $\pm$  SD.

\*: Significantly different from nonpregnant controls.  $p < 0.001$

\*\* : Significantly different from normal third trimester.  $p < 0.05$



**Fig. 4** Different dependencies of erythrocyte deformability ( $\Delta h/h$ , flow rate: 10 ml/min) on hematocrit (Hct) between normal pregnancy and nonpregnancy. The open circles indicate nonpregnancy, whereas the closed circles indicate normal pregnancy. The dextran concentrations of the suspensions were fixed at 8% for this investigation.

by changes in the hematocrit and the extracellular viscosity of the suspended medium.

Fig. 4 shows the different dependencies of erythrocyte deformability on the hematocrit value between normal pregnancy and nonpregnancy. The dextran concentration of the suspensions was fixed at 8% for this investigation. The characteristics of the curves were similar in both normal pregnancy and nonpregnancy, but the peak position of erythrocyte deformability in normal pregnancy shifted slightly to a lower hematocrit level than that in the nonpregnant control.

## Discussion

Circulating blood volume has been generally recognized to increase as pregnancy progresses. In particular, the increase of plasma volume exceeds that of erythrocyte volume, causing the blood to become diluted (7). The resulting hydremia is believed to be associated with fetal growth and development.

The increase in circulatory blood volume is often

disturbed in PIH (6). Due to the disturbance of plasma volume, the hematocrit concentration tends to be higher compared with normal pregnancy. It was reported that hematocrit was increased in severe PIH cases and that complications such as intrauterine growth retardation and fetal distress may occur (8). Thus, hemoconcentration is a clinically important feature of PIH.

The pathologic state of PIH involves vascular changes, such as hypertension and increased peripheral vascular resistance (9). Because hemorheologic changes such as hematocrit are closely related to the clinical outcomes, the hemorheologic properties have become an object of study.

Blood consists of protein-rich plasma containing highly deformable erythrocytes. Therefore, erythrocyte deformability is presumed to play an important role in regulating blood fluidity and circulation. Accordingly, measurement of erythrocyte deformability has been attempted by various methods, and filterability has been used as an index (10). However, some problems of this method were noted, as mentioned above. Therefore, we used the ESR method developed by Noji *et al.* (11).

Using ESR, it was determined that erythrocyte deformability decreased significantly in normal pregnancy as compared to nonpregnant controls. As the conditions for erythrocyte-floating suspension were fixed, the results were likely due to changes in the erythrocyte itself. The reason for such changes during pregnancy is not clear, however the administration of oral contraceptives was reported to decrease erythrocyte deformability (12). Therefore, sex hormones that increase during pregnancy may be one of the causes (13).

On the other hand, erythrocyte deformability in PIH cases showed a significantly lower value in comparison with normal pregnancy. The mechanism is not clear, however, and further study is needed.

The decrease in erythrocyte deformability may be caused by a decrease in the blood supply in microcirculation. Most of the changes during normal pregnancy are recognized as part of normal physiological adjustment. From this point of view, the decrease of erythrocyte deformability in normal pregnancy on the surface appears contradictory. However, this ambiguity may be because previous investigations were limited to changes in erythrocytes only. It was thought that extracellular viscosity (plasma viscosity) and intercellular interaction (hematocrit) were also important factors that influenced erythrocyte deformability (4). Therefore, the effect of these changes

on erythrocyte deformability were examined after changing the hematocrit and dextran concentrations in a suspended medium.

Consequently, it was found that erythrocyte deformability changed with the conditions of the suspension. Erythrocyte deformability was dependent on hematocrit, and there was the presence of an optimum hematocrit for erythrocyte deformability to exhibit the maximum value. Additionally, it was found that along with the increase of extracellular viscosity, peak shifted to a lower hematocrit level and the peak increased in value. Also, the hematocrit level where peak erythrocyte deformability occurred shifted to a slightly lower value in pregnancy compared with nonpregnancy.

Accordingly, it is speculated that the decreased erythrocyte deformability is compensated for, to some extent, by hemodilution and increased plasma viscosity in the normal course of pregnancy. On the contrary, hemoconcentration associated with PIH tended to further decrease erythrocyte deformability. The lowered erythrocyte deformability may be an important factor in the pathophysiology of PIH, such as blood stagnation and thrombopoiesis in the placenta.

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