Correlation of Serum Unconjugated Oestriol to Red Cell 2,3-Diphosphoglycerate Levels in Diabetic Pregnancy

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Summary. In order to evaluate the possible underlying factors for the increase in red cell 2,3-diphosphoglycerate content observed in late diabetic pregnancy, its relationship with serum unconjugated oestriol, human placental lactogen, haemoglobin and hydrogen ion concentrations was investigated in 42 pregnant diabetic women. A significant correlation was found between red cell 2,3-diphosphoglycerate and serum unconjugated oestriol ($r=0.54$, $p<0.001$), whereas no correlation was present between 2,3-diphosphoglycerate and the following variables: arterial pH, haemoglobin concentration and human placental lactogen. The content of 2,3-diphosphoglycerate

correlated significantly with haemoglobin-oxygen affinity expressed as P_{50} at pH 7.4 ($r=0.34$, $p<0.05$). The results of this study indicate that serum unconjugated oestriol may participate in the regulation of red cell 2,3-diphosphoglycerate content and thereby of the maternal blood oxygen release to the fetus.

Key words: Diabetic pregnancy, 2,3-diphosphoglycerate, serum unconjugated oestriol, oxyhaemoglobin dissociation curve.

It is well established that red cell 2,3-diphosphoglycerate (2,3-DPG) is an important regulator of red cell oxygen release [1, 2]. Increased levels of red cell 2,3-DPG shift the oxyhaemoglobin dissociation curve of blood to the right, thus facilitating the unloading of oxygen at the tissue level.

Red cell 2,3-DPG has been found to be increased during normal pregnancy [3–5]. In the third trimester the concentration of red cell 2,3-DPG may be elevated some 30% compared with non-pregnant women [3]. Recently we found, in a comparable study of non-smoking pregnant diabetic women, that their content of red cell 2,3-DPG was even further raised (17.8 versus 15.9 $\mu\text{mol/g}$ haemoglobin, $p<0.01$) [6].

Steroid hormones, such as hydrocortisone, aldosterone and androgens may stimulate red cell 2,3-DPG synthesis [7–9]. We therefore decided to search for a correlation of red cell 2,3-DPG with serum unconjugated oestriol, and other blood variables in the late pregnancy of diabetic subjects.

Patients and Methods

Forty-two Type 1 (insulin-dependent) pregnant diabetic women were studied in the third trimester between weeks 30–36 of gestation. The median age of the patients was 26 years (range 19–43 years) and

known median duration of diabetes was 11 years (range 3 months to 23 years). In two of the pregnant women, diabetes was diagnosed during pregnancy and both remained insulin-dependent post partum. Thirteen were classified in White class B, 14 in White class C, 13 in White class D and two in White class F. Fourteen women were non-smokers and 28 were smokers. None of the patients had severe anaemia, infections or suffered from cardiac, respiratory or renal insufficiency. The diabetes was treated according to the scheme of Pedersen [10]. The patients were given five daily meals. Thirty-seven received two insulin injections while the remaining five received one injection per day; usually a small amount of crystalline insulin mixed with NPH insulin.

Venous blood samples for determination of red cell 2,3-DPG, haemoglobin A_{1c} (HbA_{1c}), serum unconjugated oestriol, human placental lactogen, haemoglobin concentration and oxyhaemoglobin affinity were taken in the morning 30 min after the usual morning insulin dose and a light breakfast. Red cell 2,3-DPG concentration was determined by an enzymatic method according to Ericsson and de Verdier [11]. The inter-assay variation was 2.1%. Red cell 2,3-DPG was expressed as $\mu\text{mol/g}$ haemoglobin and $\mu\text{mol}/100\text{ ml}$ whole blood. The level of HbA_{1c} was determined by the macro-column chromatographic method of Trivelli et al. [12] as modified by Schwartz et al. [13]. The haemolysates were dialyzed for 18 h at 4 °C against 1000 volume of glucose-free phosphate buffer (pH 6.7). Bio Rex-70 was used as an ion exchange resin and the column was water-jacketed to maintain 25 °C. The inter-assay variation by this method in our laboratory was 2.5%. The haemoglobin concentration was measured by means of a Coulter Counter S (Coulter Electronics, Dunstable, UK). The inter-assay variation was 1.2%. Oxyhaemoglobin dissociation curves on heparinized whole blood were run in a Radiometer DCA-1 apparatus (Radiometer A/S, Copenhagen, Denmark), the principles of which

Table 1. Median values and ranges in 42 insulin-dependent pregnant diabetic women and 20 healthy pregnant women

	Healthy pregnant women	Pregnant diabetic women	
		Median	Range
2,3-DPG ($\mu\text{mol/g}$ haemoglobin)	15.9	16.8	13.7 – 20.4
2,3-DPG ($\mu\text{mol}/100\text{ ml}$)	186	217	169 – 280
pH	7.46	7.44	7.36 – 7.47
Haemoglobin ($\text{g}/100\text{ ml}$)	12.2	12.9	10.0 – 15.9
P_{50} at 7.40 (mmHg)	28.1	27.1	22.8 – 29.7
P_{50} at actual pH (mmHg)	26.7	26.2	21.8 – 29.0
Serum oestriol (nmol/l)	30	36	19 – 89
Human placental lactogen (mg/l)	6.0	6.9	4.0 – 16.6
HbA _{1c} (% of total haemoglobin)	3.9	7.3	5.0 – 9.9

Fig. 1. Correlation between serum unconjugated oestriol and red cell 2,3-DPG in 42 pregnant diabetic women in the third trimester of pregnancy ($y = 0.94x + 175$, $r = 0.54$, $p < 0.001$).

have been described by Duvellero et al. [14]. The oxyhaemoglobin dissociation curve is the characteristic sigmoid shaped curve, which appears when the fractional saturation of haemoglobin with oxygen is plotted against oxygen tension. Oxygen affinity was expressed as P_{50} (i.e. PO_2 at 50% oxygen saturation) at pH 7.4 and at actual pH using the Bohr factor -0.38 [15]. Serum unconjugated oestriol was estimated by double determinations with the commercial Amerlex Oestriol (unconjugated) radioimmunoassay kit (Amersham International, Amersham, UK). The inter-assay variation was 8%. Placental lactogen was measured in duplicate according to Nørgaard-Pedersen and Gæde [16]. The inter-assay variation was 6%.

The statistical method used consisted of a multiple regression analysis.

Results

Table 1 presents median values and ranges of the measured variables in the 42 pregnant diabetic women. For comparison, the values in 20 healthy pregnant women studied in the third trimester are also given [6]. Using multiple regression analysis, a significant positive correlation was found between red cell 2,3-DPG and serum unconjugated oestriol, both when 2,3-DPG was expressed in $\mu\text{mol/g}$ haemoglobin ($r = 0.53$, $p < 0.001$) and when expressed in $\mu\text{mol}/100\text{ ml}$ whole blood ($r = 0.54$, $p < 0.001$; Fig. 1). Keeping unconjugated oestriol constant in the regression analysis red cell 2,3-DPG did not

correlate with pH ($r = 0.26$, NS), haemoglobin concentration ($r = 0.16$, NS) or with human placental lactogen ($r = 0.002$, NS). Red cell 2,3-DPG correlated significantly with P_{50} of the oxyhaemoglobin dissociation curve at pH 7.4 ($r = 0.34$, $p < 0.05$). There was a significant correlation between human placental lactogen and serum unconjugated oestriol ($r = 0.43$, $p < 0.01$). Serum unconjugated oestriol did not correlate with HbA_{1c} ($r = 0.22$, NS).

Discussion

In the present study we found a significant positive correlation of red cell 2,3-DPG with serum unconjugated oestriol in diabetic pregnancy. The formation of serum unconjugated oestriol in the third trimester is almost entirely produced by the feto-placental unit. The finding of a positive correlation between serum level of unconjugated oestriol and red cell 2,3-DPG may suggest that oestrogenic substances influence the level of red cell 2,3-DPG. Bleicher and Pang [4] briefly commented on the possibility of such a relationship based on the observations of elevated red cell 2,3-DPG in healthy pregnant women and in women on combined oestrogen-progestogen oral contraceptives. No relationship was found between red cell 2,3-DPG, pH and the haemoglobin concentration and it is unlikely that these variables influence 2,3-DPG in this situation.

The raised level of red cell 2,3-DPG may increase the capacity of the red cell to release oxygen [17]. This is supported by our finding of a correlation between the 2,3-DPG content and the P_{50} of the oxyhaemoglobin dissociation curve. Thus the level of serum unconjugated oestriol indirectly may participate in determining maternal oxygen release to the fetus. Our observation of a relationship between 2,3-DPG and serum unconjugated oestriol may also be consistent with the finding that patients with placental insufficiency, who often show low levels of serum unconjugated oestriol, tend to have low levels of red cell 2,3-DPG [18]. From studies in vitro it has been shown that other steroid hormones may increase the level of red cell 2,3-DPG, such as hydrocortisone [7], aldosterone [8] and androgens [9]. The mechanism by which these steroids, as well as unconjugated oestriol, increase the concentration of red cell 2,3-DPG is not known.

In conclusion, we found that the increased level of red cell 2,3-DPG in late diabetic pregnancy was correlated with the concentration of serum unconjugated oestriol, suggesting that serum unconjugated oestriol may participate in determining the maternal oxygen release to the fetus in diabetic pregnancy.

Acknowledgements. This investigation was supported in part by the Northern Jutland Medical Research Fund and the Research Fund of Aalborg City Council. The technical assistance of E. Ditzel, G. Dahl Hansen, B. Søndergaard Jensen and H. Krigslund is gratefully acknowledged.

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Received: 26 April 1982
and in revised form: 14 October 1982

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