How congenital cytomegalovirus infection changes insulin and glucose homeostasis in affected fetuses

A. Ljubić, M. Cvetković, V. Šulović, M. Bujko, T. Jovanović, A. Novakov

University of Belgrade School of Medicine, Clinic for Obstetrics and Gynecology, Belgrade (Yugoslavia)

Summary

Factors affecting the fetal glucose level can be of maternal, placental or fetal origin. The level of fetal insulin during gestation is regulated by the potential of the endogenous fetal production on one hand, and on the other, by the factors (primarily glycaemia) that stimulate or inhibit its production. The aim of this paper was to analyze in which way and to what extent congenital infection with the cytomegalovirus disturbs the metabolism of fetal glucose and insulin.

Umbilical venous cord blood was obtained by cordocentesis at 22 to 29 weeks gestation from 52 women referred to our clinic for fetal karyotyping and scatological analysis of fetal CMV infection. To determine the effect of cytomegalovirus (CMV) infection on insulin and glucose fetal homeostasis, cordocentesis was performed in 18 patients (group A) with proven congenital CMV fetal infection. The control group (B) consisted of 34 patients in whom blood samples were taken for fetal karyotyping.

Maternal and fetal glucose levels were 3.95 mmol/l and 3.15 mmol/l in group A and 4.00 and 3.62 mmol/l in group B, respectively. Maternal average insulin level in group A was 14.45 mU/ml and in fetuses 10.64 mU/ml, while in group B maternal and fetal insulin levels were 12.38 mU/ml and 15.35 mU/ml, respectively. Maternal/fetal (M/F) insulin ratio was 1.35 in group A and in group B, 0.84.

Statistical analysis showed significantly lower glucose and insulin levels and also a higher maternal/fetal insulin ratio in fetuses affected by CMV infection (t = 1.4 p,0.00l). Consequences of congenital CMV infection were fetal hypoglycaemia and hypoinsulinemia.

Key words: Cytomegalovirus, Insulin, Glucose, Prenatal diagnosis.

Introduction

There are different mechanisms that regulate fetal glycaemia during gestation. While in the first half of the pregnancy the fetal glycaemia is largely dependent on the maternal glycaemia, in the second part there is much more significant control of fetal glycaemia by the feto-placental unit. At the beginning of the pregnancy the maternal and fetal glycaemia levels are almost the same, while from the 20th week of gestation there is a maternal/fetal gradient, so the fetal glycaemia level measures approximately half of the maternal value. Effective factors on glucose glycaemia levels can be of maternal, placental or fetal origin [1].

Insulin is a large polipeptide that bounds to the microfilm membrane of the placenta. On these places degradation takes place so it does not transfer to the fetus. The level of insulin during gestation, which is variable, is regulated on one side by the possibilities of endogenous fetal production and on the other, by the factors (primarily glycaemia) that stimulate or inhibit its production.

The aim of the study was to analyze in which way and to which extent congenital cytomegalovirus infection disturbs glucose and insulin metabolism.

Material and Methods

Umbilical venous cord blood was obtained by cordocentesis at 22 to 29 weeks gestation from 52 women referred to our clinic for fetal karyotyping and scatological analysis of fetal CMV infection.

To determine the effect of cytomegalovirus (CMV) infection has on insulin and glucose fetal homeostasis, cordocentesis was performed in 18 patients (group A) with proven congenital CMV infection. The control group (B) consisted of 34 patients in whom blood samples were taken for fetal karyotyping. The reason for intervention was maternal elderly age (more than 35 years). The fetal karyotype was subsequently determined to be normal in all cases. The criteria of excluding the patients from the study were EPH gestosis, Rh isoimmunisation and fetal intrauterine growth restriction. Gestational age was calculated by Naegel's rule and confirmed by an ultrasonographic scan in early pregnancy in the referring hospital-

Cordocenteses was performed as an outpatient procedure without maternal fasting or sedation. The umbilical cord vessel was identified ultrasonographically as an umbilical vein or artery by the turbulence produced after the injection of 100 to 200 microml of normal saline solution [2]. The fetal origin of the blood was subsequently confirmed by Kleinhauer Betke testing and determination of particle volume (Coulter Counter S Plus II). Maternal blood was taken from an antecubital vein immediately before fetal blood sampling.

Fetal blood was collected into heparinized syringes and blood glucose concentration was measured with a glucose oxidase analyzer (Yellow Springs Instruments), with a sample (25 micro 1) collected in a sodium fluoride tube (vacutainer, Becton, Dickinson & Co., Rutherford, N. J.). For determination of plasma insulin, 80 micro 1 of blood was centrifuged (2000 g at 4°C) and the plasma was frozen and stored at -20°C for subsequent analysis. Insulin was measured by use of the radioimmunoassay of CIS Bioindustries, Compagnie Oris Industrie SA, France. The intraassay coefficient of variation was 7.8% at 27.8% microU/ml and 6.6% at 8.0% microU/ml.

With the uniformed consent of the patient, 0.2 ml of blood

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more than for the usual procedure was taken for analysis. The additional amount of fetal blood was less than 1% of feto-placental blood volume. The project was approved by the Ethical Committee.

Statistical analysis was performed by the use of t-test and regressional analysis.

Results

There were 18 patients in group A and 34 in group B.

The characteristics of the analyzed groups are shown in the Table 1.

Maternal and fetal glucose levels were 3.95 mmol/l and 3.15 mmol/l in group A, and 4.00 and 3.62 mmol/l in group B, respectively. Maternal/fetal (M/F) glucose ratio was 1.25 in group A and in group B, 1.10. Maternal average insulin level in group A was 14.45 mU/ml and in fetuses 10.64 mU/ml, while in group B maternal and fetal insulin levels were 12.85 mU/ml and 15.35 mU/ml, respectively. Maternal/fetal (M/F) insulin ratio was 1.35 in group A and in group B, 0.84.

Statistical analysis showed significantly lower glucose and insulin levels and also a higher maternal/fetal insulin ratio in fetuses affected by CMV infection ($t = 1.14$, $p<0.001$).

Discussion

It is known that transplacental glucose transfer is by the process of facilitated diffusion, via the carriers. This transport is in direct connection with the level of maternal glycaemia until it reaches maximum saturation. The most important role is the level of the maternal glucose and the uterine blood flow, whereas placental utilization or its permeability are of much less significant importance. The factors that disable this process can lead to a change in fetal glycaemia which would alter the amount of the major energetic substances that the fetus can utilize.

Table 1. — *Characteristics of the analyzed groups*

	A (SD)	B (SD)
Age	27.4 (3.9)	31.8 (5.8)
Parity	0.4 (0.5)	1.8 (3.4)
Gestational age - cordocentesis	23.2 (3.3)	24.1 (2.8)
Gestational age - delivery	38.6 (1.1)	39.2 (0.5)

Table 2. — *Glycaemia and insulin levels in the analyzed groups*

	A (SD)	B (SD)
GLUCOSE (mmol/l)		
Mother	3.95 (0.65)	4.00 (0.37)
Fetus	3.15 (0.5)*	3.62 (0.58)
Maternal/fetal ratio	1.25 (0.08)	1.10 (0.31)
INSULIN (mU/ml)		
Mother	14.45 (3.9)	12.85 (3.4)
Fetus	10.64 (5.9)*	15.35 (4.17)
Maternal/fetal ratio	1.35 (0.6)	0.84 (0.58)**

* $P < 0.01$
** $P<0.001$

Fetal glycaemia was significantly lower in group A than in group B, while maternal glycemia was not significantly different. This implies that CMV infection lowers the fetal glucose blood level independently of other controlling mechanisms. It is possible that this infection affects the glucose metabolism in several ways. The disturbance of the carrier transport in the process of the facilitated diffusion through the placenta can be the result of CMV placentitis. A change in the permeability of the placenta is also possible, as well as more excessive placental utilization, as a result of CMV infection. One of the possible explanations of the lower fetal glycaemia levels in the cases with congenital CMV infection is a disturbance of fetal pancreas function, as a result of infection, with a consecutive change in insulin production.

Several different insulin receptors were found on the placenta. Steel *et al.* [3] suggested that a great number of placental insulin receptors show their role in glucose utilization, glycogen metabolism or lipolysis due to the analogy with the physiological effects of this hormone in other tissues. If this is accepted, then there is a question of the origin of insulin that has this effect on the placenta. Some of the more recent studies of insulin receptors on the endothelial placental cells imply the regulatory role of fetal insulin on placental metabolism [4].

It is known that the insulin level in fetal blood as well as the insulin/glucose ratio increases exponentially during pregnancy, probably as a result of the maturation of the endocrine activity of the pancreas [5]. It is thought that the fetal pancreas insulin production is relatively independent of the glycaemia until 28 weeks of gestation.

On the other hand, it has been observed that the fetal insulin level correlates very well with the maternal level. As insulin does not pass the placental barrier, this can be explained by fetal glucose dependency on the maternal glycaemia. Besides this, it has been found that there is a much higher dependency of fetal glycaemia on the level of maternal glycaemia than on the level of fetal insulinemia. This implies that the most important factor that regulates the level of fetal glycaemia is the maternal glycaemia.

Lower values of fetal insulinemia in the group with congenital CMV infection in relation to the control group correlate with the conclusion that fetal insulinemia in great part depends on fetal glycaemia. The analysis of the maternal/fetal insulinemia ratio showed a higher level in the patients in group A than in group B.

When the extent of insulin and glycaemia disturbances are compared, it can be seen that the insulin disturbance is far greater than the glycaemia disturbance. This implies that besides the glycaemia level, which to a great extent regulates fetal insulin production, there are also other factors that lead to the disturbance of its synthesis. It is possible that specific congenital CMV pancreatitis, which alters the fetal pancreatic beta cell production,

leads to the lowering of insulin production, which also affects the level of fetal glycaemia as well. The lower insulin level directs the energetic substances in other directions from the insulin sensitive tissue - skeletal muscles, liver and adipose tissue. This leads, together with hypoglycaemia to a lower glycogen level, lower fat reserves and fetal growth disturbances.

It has been concluded that congenital CMV infection leads to fetal hypoglycaemia and hypoinsulinemia.

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Address reprint requests to:

ALEKSANDAR LJUBIĆ M.D. Ph. D.
High risk pregnancy Dept.
Institute for Obstetrics and Gynecology
University clinical center of Serbia
26, Visegradska Str.
11000 Belgrade (Yugoslavia)