Cord Blood Insulin Like Growth Factor-1 (IGF-1) and its Association to Fetal Hypertrophic Cardiomyopathy in Neonates of Diabetic Mothers

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Abstract

Background: Fetal hypertrophic cardiomyopathy (HCM) is one of the well known disturbances and a well recognized comorbidity in neonates born to diabetic mothers, where intrauterine loss can occur in one third of affected fetuses and sometimes survivals can progress to overt congestive heart failure.

Insulin growth factor-1 (IGF-1) is the most important growth factor in utero and plays an important role in regulating embryonic, fetal and placental growth.

Aim of Work: Our aim was to evaluate whether there is an association between cord blood IGF-1 concentration in full term neonates and fetal HCM to explore the role of IGF-1 in the pathogenesis of septal HCM.

Material and Methods: This study was conducted on 50 neonates born to diabetic mothers and 20 control of non diabetic mothers at El Galaa Teaching Hospital.

Neonates were subjected to thorough clinical examination and echocardiographic evaluation. Cord blood IGF-1 and Maternal HbA1c were assessed.

Results: Our results revealed that 21 neonates born to diabetic mothers had septal HCM and that neonates of diabetic mothers had significant higher concentration of cord blood IGF-1 when compared to control (p=0.01). A statistically significant difference was also observed when neonates with septal HCM was compared to control and to neonates with non septal HCM (p<0.01, 0.02 respectively). A significant positive correlation was found between IGF-1 levels and interventricular septum thickness (r=0.93, p<0.001). Neonates with septal HCM were infants of suboptimally controlled diabetic mothers with positive correlation between HbA1c and interventricular septum thickness (r=0.81, p<0.001).

Conclusion: There is association between IGF-1 and fetal HCM in neonates born to diabetic mothers and that may allow modulation of the activity of IGF-1 level to be a target for therapy to modify disease progression to allow better survival in patients with childhood presentation of HCM.


Introduction

DIABETES is a common medical disorder in pregnancy. Poor control leads to increased maternal and neonatal complications. The control of hyperglycemia involves a multidisciplinary approach and is often difficult to optimise [1].

Infants of diabetic mothers are at increased risk of morbidity and mortality related to growth abnormalities (large for gestational age, small for gestational age) [2], congenital malformations, respiratory distress, hypoglycemia, hypocalcemia and iron abnormalities [3].

Chronic fetal hyperinsulinemia and accelerated fetal growth with increased amount of body fat are frequent findings in the offspring of diabetic mothers [4]. Maternal hyperglycemia results in fetal hyperglycemia because glucose readily traverses the placenta. Unchecked fetal hyperglycemia results in hypertrophy of fetal pancreatic islets and hyperinsulinemia [5]. This potent combination of hyperinsulinemia, a major anabolic hormone and hyperglycemia, a major anabolic fuel, result in a cascade of third trimester events that culminate in a striking increase in fat stores and a modest increase in protein stores [6].

Large for gestational age babies and fetal macrosomia complicate the delivery process, resulting in birth trauma due to cephalopelvic disproportion and increased risk of health problems after birth [7].
Three peptide hormones which are insulin like growth factors I, II (IGF-I, II) and insulin, seem to be the most important endocrine regulators of growth [8].

IGF-1 has a major influence on fetal and postnatal growth, it has many mitogenic actions including stimulation of cell growth, division and differentiation via specific receptors on target cell surfaces. It acts in a paracrine or autocrine level [7].

IGF-1 has also an anabolic effect, enhancing glucose and aminoacid uptake and inhibiting protein breakdown [9].

Insulin-like growth factor binding protein-1 (IGFBP-1) is the main IGF-I carrier in fetal blood which regulates fetal growth by altering the biological activity of IGF-I [3].

Cardiac malformations in infants born to diabetic mothers are five times higher than in normal pregnancies [2], those infants are at increased risk of congenital heart defects, including most commonly ventricular septal defect and transposition of the great arteries [10].

It is believed that anabolic results of fetal hyperinsulinemia triggered by maternal hyperglycemia during the third trimester can cause hypertrophic cardiomyopathy with asymmetric septum enlargement in about 30% of infants of diabetic mothers which is frequently asymptomatic until sudden unexpected cardiac death occur [11].

**Aim of the study:**

The purpose of our study was to evaluate whether there is an association between fetal hypertrophic cardiomyopathy in fetuses of diabetic mothers and cord blood IGF-1 concentration at birth in full term newborn.

**Subjects and Methods**

This study was conducted on fifty full term neonates >36 weeks of gestation born to diabetic mothers at El Galaa Teaching Hospital, during 2013 Gynecology and Obstetric Department and Admitted to Neonatal Intensive Care Unit. Exclusion criteria included: Perinatal asphyxia, preeclampsia and maternal smoking.

Twenty healthy full term neonates matched for age and sex born to non diabetic mothers with no congenital anomalies, no heart diseases, neonatal infections or systemic diseases were served as control group.

**Participants were subjected to:**

- Full medical perinatal and family history.
- Thorough clinical examination including gestational age, birth weight measurement and assessment of Apgar score.
- Laboratory investigations including:
  - Complete blood count using symnex.
  - Blood glucose levels, liver and renal profiles on Hitachi912.
  - Measurement of maternal glycated HbA1c at delivery spectrophotometrically on Biolzyer 100 as an indicator of maternal diabetic control. By using (Diasys HbA1c netFS kit, Diasys Diagnostic SystemGmbh, Holzheim).

**Test principle:**

A colorimetric enzymatic method. The concentrations of HbA1c and total hemoglobin are determined separately. After addition of R1&R2, Fructosylated dipeptides are released by protease, including the N-terminal part of the hemoglobin β-chain. Hydrogen peroxide (H2O2) is released after the oxidative cleaving of the fructosylated dipeptides by FPOX (Fructosyl peptide oxidase). The generated H2O2 is determined colorimetrically by a reaction with a suitable chromogen and the enzyme peroxidase at 660nm. The delta absorbance of the determined color is proportional to the HbA1c concentrations. Results are expressed as %HbA1c of the sample.

- Measurement of IGF-1 levels in neonatal cord blood to all cases and controls using ELISA technique according to manufacturer’s instructions (Human IGF-1 Quantikine ELISA kit, R&D systems, Inc., UK, Cat No DG 100).

4ml cord blood was withdrawn from umbilical vein at delivery in plain tube, left to clot, samples were centrifuged at 3000xg for 10min and sera obtained were separated and stored at −70 until assay.

**Test principle:**

IGF-1 Elisa kit is a solid phase enzyme linked immunosorbent assay based on the principle of competitive binding. The microtitre wells are coated with monoclonal antibody directed towards antigenic site on IGF-1 molecules. The pretreated
samples are incubated at room temperature with conjugate (biotinylated IGF-1). Wells are washed and then incubated with enzyme complex (streptavidine HRP-complex). After addition of substrate solution, intensity of color developed is reverse proportional to the concentration of IGF-1 in the samples.

- Chest radiography was performed in the first 24 hours to detect any cardiac anomalies.

- Echocardiography was performed in the first 48 hours of life to neonates of diabetic mothers to look for septal hypertrophy and to measure the myocardial thickness. Signs of obstruction and ejection fraction were also recorded. The interventricular septum (IVS) is considered hypertrophied if its thickness is >5mm at the end of diastole (IVSd) and the left ventricular posterior wall (LVPW) thickness is considered increased if >4mm. Accordingly neonates of diabetic mothers are further classified into:
  - Neonates with abnormal echo findings (n=31).
  - Neonates without abnormal echo findings (n=19).

Statistical analysis:

Data analysis was performed using SPSS windows version 10. *p*-values <0.05 was considered significant.

Results

This study was conducted on 70 full term neonates, 20 controls and 50 cases born to diabetic mothers of which 25 had gestational diabetes, 15 had type 1 diabetes and 10 had type 2 diabetes. There was no significant sex difference between males and females of diabetic mothers as regards descriptive, laboratory and echo findings (data not shown).

Table (1) shows the baseline demographic and laboratory data of the participants.

<table>
<thead>
<tr>
<th></th>
<th>Mother age (years) mean±SD</th>
<th>Parity (n) mean±SD</th>
<th>Gestational age (weeks) mean±SD</th>
<th>Birth weight (kg) mean±SD</th>
<th>Maternal HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate of diabetic mothers with abnormal echocardiography N=31</td>
<td>29.5±4.6</td>
<td>2.09±1.2</td>
<td>38.4±0.5</td>
<td>3.8±0.4</td>
<td>8.0±0.3</td>
</tr>
<tr>
<td>Neonate of diabetic mothers without abnormal echocardiography N=19</td>
<td>28.9±5.7</td>
<td>2.1±0.9</td>
<td>38.7±0.6</td>
<td>3.6±0.5</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>Neonates of non diabetic mothers (control group) N=20</td>
<td>28.5±6.2</td>
<td>2.0±1.1</td>
<td>37.8±0.8</td>
<td>3.2±0.6</td>
<td>5.4±0.7</td>
</tr>
</tbody>
</table>

Table (2) reveals the echocardiographic findings in neonates of diabetic mothers, where 21 mothers were found to have infants with neonatal septal hypertrophic cardiomyopathy range from (6-10.5mm).

Table (3) & Fig. (1) reveal that infant born to diabetic mothers had significantly higher concentration of cord blood IGF-1 when compared to control (*p*=0.01). There was also a statistical significant difference in IGF-1 between neonates of diabetic mothers with septal hypertrophic cardiomyopathy when compared to control and to neonates with non septal hypertrophy (*p*<0.01, 0.02 respectively).

Fig. (2) shows correlation study between IGF-1 and interventricular septum thickness in neonates of diabetic mothers where a significant positive correlation was found (*r*=0.93, *p*<0.001).

Fig. (3) shows correlation study between HbA1c and interventricular septum thickness in neonates of diabetic mothers where positive linear correlation was found (*r*=0.81, *p*<0.001).
Table (2): Echocardiographic findings in neonates of diabetic mothers.

<table>
<thead>
<tr>
<th>Echocardiographic findings</th>
<th>Number of cases</th>
</tr>
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<tbody>
<tr>
<td>Septal hypertrophic cardiomyopathy</td>
<td>21 (42%)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Ventricular septal defect</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>No abnormality detected</td>
<td>19 (38%)</td>
</tr>
</tbody>
</table>

Table (3): Comparison of IGF-1 levels among neonates of control mothers and diabetic mothers with and without septal hypertrophic cardiomyopathy.

<table>
<thead>
<tr>
<th>IGF-1 (ng/ml) mean±SD</th>
<th>gp A versus control</th>
<th>gp A versus gp B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates of non diabetics (control)</td>
<td>49.7±6.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Neonates of diabetic mothers</td>
<td>65.6±16.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group A: Neonates of diabetics with septal hypertrophy n=21</td>
<td>80.3±4.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Group B: Neonates of diabetics without septal hypertrophy n=29</td>
<td>60.1±11.8</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Insulin like growth factor-1 (IGF-1) plays a pivotal role in regulating embryonic, fetal, and placental growth. It has a short term insulin like metabolic effects and long term growth factor like effects on cell proliferation and differentiation of various cell types [8].

Evidence suggests that maternal IGF-1 which does not cross the placenta could influence fetal growth by regulating placental transfer of nutrients to fetus, in turn these nutrients could increase fetal IGF-1 secretion and bioavailability and thus promote the fetal growth [12].

Infants of diabetic mothers have long been recognized to be at risk of having hypertrophic cardiomyopathy, a condition that is characterized by unexplained thickening of the interventricular septum.
septum and ventricular walls and by systolic and diastolic dysfunction of the neonatal heart in the absence of hemodynamic stress [13].

Although the underlying mechanism for developing this condition is still unknown and septal hypertrophy commonly regress spontaneously, those infants are still prone to progression of the condition to ischemic cardiomyopathy or sudden cardiac death earlier in life than in other normal infants [1].

IGF-1 is known to mediate many, if not most, of the anabolic effects of circulating growth hormones including those in the heart [14].

In our study, we found that cord blood IGF-1 is statistically significantly higher in neonates of diabetic group than in the control group, (p=0.01) and in our 21 cases of neonatal septal hypertrophic cardiomyopathy born to diabetic mothers, the level of IGF-1 is statistically significantly higher when compared to control mothers and to non septal hypertrophy group (p<0.01, 0.02 respectively).

Similar to ours, Hyati et al. [1], had detected 6 cases (out of 50 cases with diabetics) with neonatal septal hypertrophic cardiomyopathy and when serum IGF-1 concentration were measured in maternal blood at delivery, they were significantly higher in diabetics group than control and highly significant in group of neonates with septal hypertrophy when compared to control (p<0.001). They postulated that the extremely high maternal serum IGF-1 concentration during delivery in the six mothers whose babies had neonatal septal hypertrophy suggests that, high concentration of IGF-1 in late pregnancy play a role in the development of this condition.

Marian [15] has found in his study that hypertrophic cardiomyopathy caused by excess insulin and IGF-1 in neonates of diabetic mothers is associated with reduced rather than increased ejection fraction. They had postulated that IGF-1 is upregulated due to increased IGF-1 receptor expression in various tissue including heart and that elevated neonatal IGF-1 is the major contributor to the pathological manifestations of HCM.

In accordance also to our results, Gonzaler et al. [16], have found 11 neonates (34%) out of 57 born to diabetic mothers have HCM and that cord serum IGF-1 was statistically significantly elevated in fetuses of diabetic mothers compared to control and in HCM group compared to non HCM and control. They concluded that this elevated neonatal IGF-1 is associated with fetal HCM in fetuses of diabetic women and is the major accuser to pathologic heart development in fetal life.

In an attempt to evaluate the relations between cord serum IGF-1 and HCM, correlation studies revealed a highly significant positive linear correlation (r=0.93, p<0.001). Similarly, Dawid et al. [8], reported a positive association between cord blood IGF-1 levels and both ventricular end diastolic dimensions of interventricular septum. Creszynska et al., [17] also reported linear association between cord blood IGF-1 and both right and left ventricular dimensions during end diastole (r=0.57, 0.46, p<0.001, 0.01 respectively). A similar trend was observed by El Ganzoury et al. [3], who reported a positive correlation between cord blood IGF-1 and left ventricular posterior wall thickness and left ventricular end diastolic dimensions (r=0.38, 0.546, p<0.001, 0.0001 respectively) in newborns of diabetic mothers. These findings can confirm our speculations that IGF-1 is most likely playing an important role in the development of cardiac morphological and functional changes in neonates of diabetics.

Another finding worth discussing in the current study is the significant positive correlations between maternal HbA1c and interventricular septum thickness (r=0.81, p<0.001) and most of them were large for gestational age >90th percentile. Our study coincides with the study done by El Ganzoury et al., [9] Where thirty neonates (43.5%) had hypertrophic cardiomyopathy (HCM), all of them were infants of suboptimally controlled diabetic mothers (HbA1c >7%) with positive correlation between HbA1c and interventricular septal (IVS) thickness. Cooper et al., [18] found a strict correlation between occurrence and degree of HCM with metabolic control during the third trimester. On the contrary, Rizzo et al., [19], found an accelerated increase in cardiac size in fetuses of diabetic mothers, in spite of a careful metabolic control. Franzese et al., [20] also reported a case of severe hypertrophic cardiomyopathy in large for gestational age neonates born from well controlled diabetic mother.

In conclusion, HCM is a well recognized complications in neonates of diabetic mothers and its outcome is highly variable, with some remaining asymptomatic, some developing symptoms and all remaining at risk for progressive heart failure and fetal or neonatal deaths. Our findings supported by previous studies provide an emphasis on the association between IGF-1 and fetal HCM in neonates born to diabetic mothers. They provide a testable hypothesis in which high IGF-1 levels either contributes to or permits the occurrence of
fetal HCM either through endocrine control or an overspill from mechanisms at the cellular level remain to be determined. If this hypothesis proves true, modulation of the activity of IGF-1 at either IGF-1 Receptor level or IGF-1 Binding Protein level may be a target for therapy to modify disease progression to allow better survival in patients with childhood presentation of HCM [21].

However, further studies are still warranted on large size populations to establish the exact role for IGF-1 in the pathogenesis of HCM.

Our recommendation allows for preconceptional testing of HbA 1c in high risk populations to identify women at risk for abnormal glucose homeostasis and early screening before 24 weeks of gestation to detect gestational diabetes as those patients should be strictly followed in the periconceptional period for tight glycemic control to reduce the incidence of abnormal organogenesis in pregnancy.

References