SOME METABOLIC CHANGES IN NEWLY BORN PUPPIES OF DIABETIC BITCHES

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ABSTRACT
The present study is designed to explain some of the major metabolic complications in puppies of diabetic bitches. For this aim, twelve newly born puppies (n = 12) of six pregnant diabetic bitches and six non-diabetic ones were collected in El-Bayda pet animals clinic at Omer El-Mokhtar University - Faculty of Veterinary Medicine – Republic of Libya. Venous blood samples were collected from each puppy during the 24 hours after birth. The present data revealed hypoglycemia, hyperinsulinemia, hyperlactacidemia, hypocalcemia, hypomagnesemia, hyperbilirubinemia. Also, a significant increase was observed in hepatic function enzymes [alanine aminotransferase (ALT), asparate aminotransferase (AST) and gamma glutamyl transferasre (GGT)], alkaline phosphatase (ALP) and hematocrit values (PCV) in puppies of diabetic dams on comparison with the mean values of the puppies of non-diabetic ones. On the other hand, manganese (Mn), zinc (Zn) and copper (Cu) levels were non-significantly changed. Moreover, acid phosphatase (ACP), urea, creatinine and plasma immunoglobulins (IgG, IgA and IgM) concentrations were non-significantly changed. This study highlights the great risk of maternal diabetes on the newly born puppies.
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**INTRODUCTION**

Diabetes mellitus (DM) is a state of chronic hyperglycemia. Many factors have been involved in the pathogenesis of DM including environmental, immunological and genetic. These factors may cause hyperglycemia by reducing endogenous insulin or by opposing its action. The lack of insulin leads to abnormalities of carbohydrate, protein and lipid metabolism. The increased incidence of DM that is complicating pregnancy is of concern since it is associated with an increase in mortality and morbidity of the fetus and neonate (30). Despite the current improvement of diabetes care in pregnancy, neonatal complications are still more frequent than in the general population (19). Many of these complications are related to the severity of the maternal hyperglycemia during pregnancy (29) and to the metabolic status of the diabetic mother (25).

Diabetes in pregnant females can be detrimental to her fetus for many reasons. First, she has an increased spontaneous abortion rate (17). Second, 6 % to 8 % prevalence of major congenital anomalies has been found in the newborns of diabetic dams mainly cardiovascular, central nervous and musculoskeletal systems (15).

The major negative consequences of neonates of diabetic mothers are macrosomia, neonatal morbidities and respiratory distress syndrome (12), hypoglycemia (20), hyperbilirubinemia, disturbed hepatic function and hypocalcemia (30) and cardiomyopathy which is secondary to the anabolic effect of fetal hyperinsulinemia (6).

Accordingly, the aim of the present investigation is to study some of the major metabolic changes that may occur in the newly born puppies of diabetic bitches after birth.

**MATERIALS AND METHODS**

**Animals :**

Twelve newly born puppies of six pregnant diabetic bitches and six non-diabetic ones were admitted at El-Bayda pet animals clinic at Omer El-Mokhtar University - Faculty of Vetrinary Medicine – Republic of Libya. Diabetes mellitus in bitches was diagnosed on the basis of the clinical signs and determination of fasting blood glucose level and insulin level. The major clinical signs observed were polyuria, polydipsia, obesity and cataract. All diabetic bitches had hyperglycemia, glucosuria and hypoinsulinemia.
**Samples**

Venous blood samples were collected from each puppy during the first 24 hours after birth. Blood samples were withdrawn by a vein puncture from the cephalic veins according to Kirk and Bistner (13). The blood samples were divided into 2 portions, the first on EDTA for whole blood analysis, the other one was allowed to coagulate at room temperature and then the clear sera were separated by aspiration after centrifugation at 3000 rpm for 10 minutes.

Analysis of Sera and Whole Blood Samples: The collected sera were freshly used for quantitative determination of glucose (2), lactate (28), Insulin (18), calcium (10), magnesium (4), total bilirubin (14), urea (21), creatinine (11), immunoglobulin concentration (16), gamma glutamyl transferase (GGT) [EC 2.3.2.2] (22), alanine amino transferase (ALT) [EC 2.6.1.2], asparate amino transferase (AST) [EC 2.6.1.1], and alkaline phosphatase (ALP) [EC 3.1.3.1] (3), acid phosphatase (ACP) [EC 3.1.3.2] (32) and trace elements (Zn, Cu, and Mn) by using 5Pg atomic absorption spectrophotometer (Pye Unicum) model 3300 USA according to Fernandy and Kahen (9). The whole blood EDTA samples used for determination of Packed cell volume (PCV) (7)

**Statistical Analysis:** The obtained data were statistically analyzed and the significant difference between groups was evaluated by t-test as explained by Snedecor and Cochran (27). All differences were considered significant at p<0.05.

**RESULTS**

The recorded data in the provided tables revealed significant increases (P<0.05) in the mean values of serum insulin, PCV % (table 1), total bilirubin, AST, ALT and GGT (table 3) in the experimental group (newly born puppies of diabetic dams) compared to the control group (newly born puppies of non-diabetic dams). Moreover, there was a highly significant increase (P<0.01) in serum lactate (table 1) and ALP (table 3) in the experimental group compared to control group.

On the other hand, there were significant decreases (P<0.05) in the mean values of serum glucose (table 1), calcium and magnesium (table 2) of the experimental group (newly born puppies of diabetic dams) compared to the control (newly born puppies of non-diabetic dams).

Meanwhile the reported data showed non-significant increases in serum copper (table 2), ACP (table 3) and IgA (table 4); and non-significant decreases in serum zinc, manganese (table 2), IgG and IgM (table 4) of the experimental group (newly born puppies of diabetic dams) compared to the values recorded in the control (newly born puppies of non-diabetic dams).
Table 1: The mean values ± S.E of Glucose, Lactate, Insulin and PCV% in newly born puppies of non-diabetic and of diabetic dams.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg / dl)</th>
<th>Lactate (mg / dl)</th>
<th>Insulin (µ IU/ml)</th>
<th>PCV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (puppies of non-diabetic dams)</td>
<td>79.80 ± 1.2</td>
<td>41.90 ± 1.09</td>
<td>9.33 ± 0.21</td>
<td>31.91 ± 2.60</td>
</tr>
<tr>
<td>Experimental (puppies of diabetic dams)</td>
<td>50.03 ± 1.09*</td>
<td>69.30 ± 2.11**</td>
<td>18.39 ± 1.07*</td>
<td>55.18 ± 3.11*</td>
</tr>
</tbody>
</table>

* Significantly different at P < 0.05
** Highly significantly different at P < 0.01

Table 2: The mean values ±S.E of Calcium, Magnesium, Zinc, Copper and Manganese in newly born puppies of non-diabetic and of diabetic dams.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium (mg / dl)</th>
<th>Magnesium (mg / dl)</th>
<th>Zinc (µg/dl)</th>
<th>Copper (µg/dl)</th>
<th>Manganese (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (puppies of non-diabetic dams)</td>
<td>9.30 ± 0.87</td>
<td>2.66 ± 0.16</td>
<td>19.20 ± 0.50</td>
<td>26.33 ± 1.10</td>
<td>8.10 ± 0.18</td>
</tr>
<tr>
<td>Experimental (puppies of diabetic dams)</td>
<td>6.21 ± 0.83*</td>
<td>1.08 ± 0.13*</td>
<td>18.6 ± 0.61</td>
<td>27.11 ± 1.40</td>
<td>7.75 ± 0.20</td>
</tr>
</tbody>
</table>

* Significantly different at P < 0.05

Table 3: The mean Values ±S.E of total bilirubin, AST, ALT, GGT, ALP and ACP in newly born puppies of non-diabetic and of diabetic dams.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total bilirubin (mg / dl)</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>ACP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (puppies of non-diabetic dams)</td>
<td>0.96 ± 0.03</td>
<td>18.40 ± 0.93</td>
<td>22.11 ± 1.16</td>
<td>7.30 ± 0.69</td>
<td>1.17 ± 0.08</td>
<td>0.69 ± 0.02</td>
</tr>
<tr>
<td>Experimental (puppies of diabetic dams)</td>
<td>2.63 ± 0.26*</td>
<td>38.61 ± 2.10*</td>
<td>47.16 ± 2.22*</td>
<td>17.11 ± 1.11*</td>
<td>7.15 ± 1.02**</td>
<td>1.05 ± 0.11</td>
</tr>
</tbody>
</table>

* Significantly different at P < 0.05
** Highly significantly different at P < 0.01
Table 4: The mean values ± S.E of immunoglobulins (IgA, IgG and IgM) in newly born puppies of non-diabetic dams and of diabetic dams.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IgA (mg/dl)</th>
<th>IgG (mg/dl)</th>
<th>IgM (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (puppies of non-diabetic dams)</td>
<td>63.25 ± 2.11</td>
<td>261.40 ± 6.11</td>
<td>45.37 ± 2.13</td>
</tr>
<tr>
<td>Experimental (puppies of diabetic dams)</td>
<td>71.60 ± 2.90</td>
<td>250.19 ± 4.12</td>
<td>39.11 ± 2.71</td>
</tr>
</tbody>
</table>

DISCUSSION

The recorded data revealed that there was hypoglycemia in the experimental group which coincided with the results obtained by Amina et al. (1) and Cordero et al. (5) who stated that hypoglycemia has been observed in newly born infants of diabetic mothers. The detected hypoglycemia could be attributed to the hypertrophy and hyperplasia of the Langerhans islets, which might be connected with insulin resistance in pipheral tissues as a significant risk associated with diabetic mother in pregnancy (8). This result was also confirmed by the opinion of Rakhab and Chernev (24) who proved that the hypoglycemia of the infants of diabetic mothers is probably due to the high insulin levels caused by the hyperplasia of the pancreatic beta-cells that was normally found in these infants. The observed hyperinsulinemia was similar to the recorded data in infants of diabetic mothers by Oberhoffer and Miodovnik (19) who showed a proportional increase in serum insulin with maternal glycated hemoglobin in them, it was also stated by Simmons (26) that the hyperinsulinemia depended on the degree of maternal hyperglycemia which might be related to the primary source of glucose in the early postnatal hours is mobilized from the hepatic glycogen with appropriated catecholamins and glucogen response however this response is blunted in diabetic mothers. Furthermore the conversion of triacylglycerol to fatty acids and glycerol which is ultimately converted to glucose is prevented because of the high insulin and low catecholamine and glucagon levels in the circulation (30).

Regarding the highly significant elevation of the serum lactate level, the presented data agreeded with the results of Pribylova and Dovrakova (23) who found a positive correlation between maternal glycated hemoglobin and plasma.
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lactate in all diabetic females and their infants. The recorded hyperlactacidemia might be attributed to that the maternal hyperglycemia resulted in fetal hyperinsulinemia thereby, increasing metabolic rate and oxygen demand which exceeds oxygen availability leading to fetal hypoxia and increase lactic acid (1).

The recorded hypocalcemia, hypomagnesemia and increased ALP activity were similar to the results observed by Rakhab and Chernev (24) who found a negative correlations between maternal hyperglycemia and serum calcium, magnesium and the activity of serum ALP. These changes could be probably secondary to the transient functional hypoparathyroidism that might be also secondary to the maternal and fetal hypomagnesemia.

The recorded significant increase in serum total bilirubin and the activities of the hepatic function enzymes AST, ALT and GGT indicated the direct evidence for fetal hypoxia and polythecyemia which are hepatic stressors as stated by Ylevin et.al. (31) who observed that the polythecyemia resulted from increased hemoglobin which then increase bilirubin due to the elevated turnover of heme and decrease clearance of bilirubin. These changes could also be related to the increased hemolysis, elevated red cell masses, ineffective erythrobiosis, prematurity, increased bruising and trauma and retardation of enzymesystem maturation (29).

The regarded sigificant increase in PCV % was similar to the data recorded by Cordero et.al. (5) who stated that there were a significant increase in Hb, PCV, and RBCs in infants of diabetic mothers which might be attributed to the fetal hypoxia and polycytheemia.

The detected non-significant changes in serum IgA, copper, ACP, IgG, IgM, zinc and manganese suggest that these changes could be independent on the maternal diabetes, which means that maternal diabetes of bitches is not associated with changes of these parameters in newly born puppies.

In conclusion, this study provided an insight on the great risk of maternal diabetes of bitches on their newly born puppies. Therefore, it is recommended that the thorough control of diabetes mellitus in pregnant bitches is crucial in order to avoid the short-term complications in the newly born puppies.

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106.
Some of the changes in the skin of newborn puppies of mothers with diabetes \m. Hussein Abdel-Maksoud*, Yaqout Abdel-Fattah Al-Sunusi*, Mohammed Mohamed Gamal**, Department of Veterinary Chemistry and Animal Pathology, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Diabetic mothers are considered a risk factor for the development of cutaneous changes in newborn puppies. The aim of this study was to investigate the effect of diabetes on the skin of newborn puppies. The study was conducted on 24 newborn puppies (12 diabetic and 12 healthy) from diabetic mothers. The skin was evaluated for changes in color, texture, and exudate. The results showed that newborn puppies of diabetic mothers had more severe skin changes compared to healthy puppies. The study also investigated the immune system changes in diabetic mothers, and no significant differences were found. The study concluded that diabetic mothers are a risk factor for skin changes in newborn puppies.