Effects of Maternal Diabetes on the Structure of Cervical Segments of the Spinal Cord in the Developing Fetus

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Diabetic pregnancy represents both a clinical and research challenge in terms of its detrimental effects on the foetus. Previous studies have suggested that maternal diabetes mellitus may cause lasting effects on the psychoneurological development in the offspring. Thus, the present study is aimed to observe structural changes in the spinal cord of developing foetus of diabetic mouse mother. A total of 124 adult mice (100 females and 24 males) of ICR strain were used. Diabetes was induced in 55 female mice by two intraperitoneal injections of streptozotocin. Animals with blood glucose level > 200 mg dL were considered diabetic and mated with adult male mice. Another 45 female mice served as controls without any diabetes induction. Successful mating in treated and control animals was indicated by the presence of vaginal plug and this day was considered as gestational day (GD) 0. Pregnancy was terminated on GD 14, 16, 18 and 20 (day of delivery). Foetuses and pups were fixed in 10% formaldehyde for light microscopy study. The light microscopical observation demonstrated bilateral asymmetry of the two-halves of the cervical segments of the spinal cord. Shrunken or eroded dorsal horn represented the most frequent defect in foetus/pup of GD 16 (n=23), GD 18 (n=21) and GD 20 (n=26). In addition, the white matter on the lateral and dorsal side has much reduced or disappeared. Imperfect growth and protrusion of spinal cord were also observed. In some cases, the dorsal part of the spinal cord has protruded beyond the vertebral lamina; showing a condition called meningocele. Irregularity and dilatation of central canal were noticed. These changes were not present in the control samples. These findings implicated that the central nervous system is subjected to structural changes in the developing foetus when exposed to diabetic milieu. The results are supportive of the previous investigations in the human that indicated neurobehavioural and habituation disturbances in the offspring of diabetic mothers.

Key words: Maternal diabetes, developing fetus, spinal cord structure

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Introduction

The escalating incidence and prevalence of diabetes mellitus have placed this debilitating disease as one of the major threats to the global public health. According to the previous reports, the prevalence of diabetes mellitus was found to be 8.2% of general population and is increasing by the tick of time. Congenital malformations are one of the leading causes of perinatal mortality among children of diabetic mothers (Martin et al., 1987). Despite of the improved prenatal diabetic management and neonatal care, the incidence of congenital malformations caused by maternal diabetes has not decreased significantly over the years (Molsted-Pedersen, 1980). Generally, the incidence of congenital malformations is two- to five-fold higher compared to non-diabetic pregnancy (Miller et al., 1981; Becerra et al., 1990). Malformations are expressed through a wide variety of organs and body systems but most common birth defects are those of central nervous system and limbs (Cockroft and Coppola, 1977). Neonatal hypoglycaemia caused by maternal diabetes poses a detrimental effect on the central nervous system. It is beyond doubt that children born to diabetic mothers experience neurological dysfunction such as mental retardation, gross and fine motor deficits, longer habituation period, epilepsy, hemiplegia, impulsiveness and hyperactivity (Cummins and Norrish, 1980; Stenninger et al., 1998; Ornoy et al., 1999; Hod et al., 1999; Doherty and Hepper, 2000).

Histopathological changes were observed in the neural folds of exencephalic embryos exposed to high glucose level and β-hydroxybutyrate (Sadler, 1980; Horton and Sadler, 1983). The exencephalic embryos exhibited open neural folds in the cranial region and some with widespread, elevated folds but did not fuse completely. Pyknotic debris was seen but it was not of significant amount. In a different experiment, cytoplasmic “vacuoles” were indicated in the neuroepithelium of mouse embryos exposed to β-hydroxybutyrate (Horton and Sadler, 1983). This striking histological feature was actually highly amplituded-swelled mitochondria when observed under electron microscope.

Most of the previous studies that were done on the effect of maternal diabetes on the foetus focused more on the gross morphological malformations. Histopathological changes were observed only on the early stage of neural tube development (Sadler, 1980; Horton and Sadler, 1983) but not on the later stages which results in the spinal cord and brain. No particular study aiming to evaluate and analysing the cause of neurological abnormality is conducted. To date, there is no known study done on this particular subject as verified from available literatures.

The objectives of this study are

a) to observe the histological changes of the spinal cord in the developing foetus of diabetic mouse mother.

b) to relate the possible functional significance of the changes in the spinal cord.

Materials and Methods

A total of 124 adult mice (100 females and 24 males) weighing 25.0 g to 30.0 g of ICR strain (purchased from the Institute of Medical Research) were used. They were of similar genetic and
environmental background. Fifty-five female mice were used for induction of diabetes mellitus whereas forty-five were used as controls (normal subjects without diabetes induction). The adult males were used for mating. The mice were allowed 1 week to acclimatise to the room conditions before the start of the study. They were kept at room temperature (26°C to 35°C) on an approximately 12 h light/12 h dark cycle with free access to food and raw water. Cages and bedding (wood shavings) were cleaned 2 times weekly.

**Induction of Diabetes Mellitus**

Each adult female mouse was given one intraperitoneal injection of streptozotocin (Sigma SO 130) (50 mg kg\(^{-1}\) body weight) dissolved in 0.1 M citrate buffer (pH = 4.0) and the second dose was injected one week later. Anaesthesia with diethyl ether was done before the injection. One week after the second injection, the blood glucose level (obtained through tail-cut) was measured by glucometer (Precision Q, I, D\(^{6}\) Blood Glucose Monitoring System, Medisense\(^{8}\), Abbott). Animals with glucose concentration ≥ 200 mg d\(^{-1}\)L (≥ 11.1 mgd L\(^{-1}\)) were considered diabetic and used in the experiment. The control female mice blood glucose levels were checked to ensure that they were not diabetic. The weight of the mothers was measured by electronic weighing machine and was recorded every two days.

**Estrous cycle determination**

Before the female mice were mated, cervical smears were done to determine the estrous cycle. Small cotton bud was inserted into the vagina of the female and was twisted in one direction to slough off the epithelial lining. The sample of epithelial cells was smeared on a microscopic slide, air-dried and stained with methylene blue. Later, the slide was cleaned by running water and air-dried before being examined under light microscope. The presence of cornified cells indicates estrous stage and females with this feature are ready for mating for successful pregnancy.

**Mating**

The mice were assigned to cages (15x28x38 cm) with the ratio of two males to one female for successful mating. Both the control and diabetic female mice were mated overnight and the presence of a vaginal plug on the next morning was regarded as gestational day 0 (GD 0). They were then removed to respective cages (control and diabetic mice) with other pregnant females of the same date of gestational day. Both control and diabetic female mice blood glucose levels were checked during pregnancy.

**Tissue preparation**

Pregnancy was terminated on GD 14, GD 16, GD 18 and on the day of delivery (GD 20). The pregnant mice were euthanised with an overdose of diethyl ether and foetuses were recovered from the uterine horns through caesarean section. Full-term pups were taken after delivery and sacrificed with an overdose of diethyl ether. The number of foetuses and pups were recorded.
Tissue processing for light microscopy

The tissues were fixed in 10% formaldehyde for 7 days. The foetuses and pups were trimmed below the ears at the upper cervical region and above the lower limbs, leaving the trunks. They were then segmented into 2 to 3 segments and placed into the tissue cassettes. The cassettes were put into the tissue basket of the automatic tissue processor (LEICA TP 1020). The tissue specimens in the tissue basket were automatically switched from one chemical to another for a duration of 16 h for dehydration, clearing and infiltration by wax. The sequence of chemicals was as follow: rinsing with different grades of alcohol (70, 80, 90, 95%), dehydration with absolute alcohol (100%), clearing with chloroform and eventually impregnation in paraffin wax. Then, the tissues were embedded with paraffin wax (BDH®, melting point 58 °C) to form tissue blocks in the paraffin embedding centre (LEICA EG 1160). Five microns thick serial sections were taken with a semi-motorized rotary microtome (LEICA RM 2145). The sections were floated in hot water bath, mounted on adhesive-coated glass slides and dried on slide warmer (LEICA HI 1210). The sections were stained by Haematoxylin-Eosin.

Statistical analysis

SPSS Data Processing Version 10.0 was used for statistical analysis.

Results

Diabetic female mice parameters

In the control female mice, 20 out of 45 were confirmed pregnant (Table 1).

In the experimental subjects, 24 deaths were noted during diabetes induction. These deaths occurred immediate to or a few days after the first or second dose of intraperitoneal injections.

<table>
<thead>
<tr>
<th>Group</th>
<th>No of mothers</th>
<th>Maternal weight gain</th>
<th>No of fetuses / pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD 14</td>
<td>5</td>
<td>1.25</td>
<td>64</td>
</tr>
<tr>
<td>GD 16</td>
<td>4</td>
<td>1.41</td>
<td>52</td>
</tr>
<tr>
<td>GD 18</td>
<td>5</td>
<td>1.75</td>
<td>65</td>
</tr>
<tr>
<td>GD 20</td>
<td>6</td>
<td>2.12</td>
<td>73</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td></td>
<td>254</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GD 14</td>
<td>4</td>
<td>1.26</td>
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<td>GD 20</td>
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<td>52</td>
</tr>
<tr>
<td>TOTAL</td>
<td>16</td>
<td></td>
<td>190</td>
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</tbody>
</table>
Fig. 1: Normal structure of the spinal cord on day 14 of development (Control)

Fig. 2: Normal structure of the spinal cord on day 20 of development (Control)

of streptozotocin. Immediate deaths might be due to overdosage of anaesthesia or internal haemorrhage caused by improper injection method whereas deaths after few days might be caused by diabetic ketoacidosis. Out of 31 streptozotocin-induced diabetic female mice, only 16 were pregnant successfully.
Fig. 3: Structure of the spinal cord on day 16 of development (Experimental) Shows damage to the lateral funiculus (left side) and adjacent structures

Fig. 4: Structure of the spinal cord on day 16 of development (Experimental) Shows damage to the lateral funiculus (right side), part of dorsal Funiculus etc
Maternal and foetal parameters

The streptozotocin-induced diabetic mothers demonstrated a maternal weight gain (+ foetus) during gestational day 14 and 16 which was slightly greater compared to the non-diabetic animals but it was not significant.

Maternal blood glucose level parameters

Control mothers’ blood glucose level ranged from 5.0 mmol L⁻¹ to 10.5 mmol L⁻¹. However, the range of treated female mice blood glucose level was 11.5 mmol L⁻¹ to 25.4 mmol L⁻¹, thus confirming that they were diabetic (≥ 11.1 mmol L⁻¹).

Foetal spinal cord structural changes

Light microscopical observations on the spinal cord of the developing foetuses/pups of diabetic mothers revealed of 5 major groups of structural changes, namely asymmetry of spinal cord, dorsal horn defects, spinal cord protrusion, funiculus defects and underdevelopment of the spinal cord. The proportion of these defects varied in different gestational days. Highest incidence of dorsal horn defects was noted on GD 20 (n=26), GD 18 (n=21) and GD 16 (n=23). In GD 14, the changes were minimal.

Structure Of The Cervical Segments Of The Spinal Cord (Normal Structure) In Foetus/pup Born To Control Mothers

Gestational Day 14 [Fig. 1]

The spinal cord at cervical regions appears well-developed with the complete fusion of the neural folds and has fully occupied the vertebral canal. However, the central canal is large and elongated. The shape of the cord is almost oval with bilateral symmetry. Prominent grey and white matters are observed.

Gestational Day 16

During this period, the most striking differential feature is the smaller and less elongated central canal. The spinal cord shows bilateral symmetry and has fully occupied the vertebral canal.

Gestational Day 18

The cervical segments of the spinal cord is well-developed as indicated by the defined organisation of the grey and white matters. The central canal is significantly smaller and less elongated. Overall, the two halves of the spinal cord are oval and symmetrical. The vertebral canal is fully occupied by the spinal cord, leaving little space in between the spinal cord and the vertebrae.
Gestational Day 20 [Fig. 2]

By this age, the spinal cord has developed sufficiently. The grey and white matters have distinct structures with proper arrangement. Prominent dorsal and ventral horns are seen and they are surrounded by the ventral, lateral and dorsal funiculi. As noted in the previous gestational age (Fig. 1), the spinal cord shows bilateral symmetry and has fully occupied the vertebral canal.

Histopathological Changes Of The Spinal Cord In Foetus/pup Born To Diabetic Mothers

Gestational Day 14
Cervical Cord:
No significant changes are observed at this level. The spinal cord appears normal.

Gestational Day 16 [Fig. 3 and 4]

The lateral funiculus is eroded at one or both sides, thus ensuing asymmetry of the two-halves of the cord. The dorsal horn is also reduced in size. In some cases, a large gap is visible posterior to the cord, remaining within the meningeal layers. The central canal is normal.

Gestational Day 18 [Fig. 5]

There is asymmetry of the spinal cord. In some fetuses, distinct defects in the dorsal funiculus are noticed. This includes splitting or erosion of the dorsal column. Accompanying such structural changes are dorsal horn defects and elongated space posterior to the spinal cord. Some segments of the cervical cord appear less developed or imperfectly developed as indicated by the large space around the cord. There is posterior protrusion of the cervical cord that reached the lamina of the vertebra. Split dorsal column is also noted. However, the central canal appears to be normal.

Gestational Day 20 [Fig. 6, 7 and 8]

The spinal cord has herniated into a sac comprising the meninges and protruded through the bone defect (Fig. 6). This defect is meningomyelocele. The lateral funiculus displays shrinkage and irregularity and the grey and white matters are disorganized, thus leading to bilateral asymmetry and irregularity of the vertebral canal. These defects are apparently relatively more frequent and dominant on the right side of the cord. In some instance (Fig. 7), the dorsal part of the spinal cord is enlarged and extended onto the right or left side. In some instance (Fig. 8), in addition to the bilateral asymmetry, dorsal projection, central splitting of the cord etc, the epimysium of the dorsal cervical muscles is closely applied onto the surface of the spinal cord, with imperfect development of the dorsal bony laminae of the vertebrae and possibly with an imperfect development of the meningeal layers of the spinal cord. Large space around the spinal cord is also noticed in most cases. However, central canal looks normal.
Fig. 5: Structure of the spinal cord on day 18 of development (Experimental) Shows asymmetry, imperfect development of the dorsal funiculus, central splitting etc.

Fig. 6: Structure of the spinal cord on day 20 of development (Experimental) Shows asymmetry, irregularity, imperfect development, shrinkage, disorganized gray and white matters, dorsal protrusion / herniation from the right half of the spinal cord (meningomyelocele) etc.
Fig. 7: Structure of the spinal cord on day 20 of development (Experimental) Shows enlargement and projection of the spinal cord on the right side.

Fig. 8: Structure of the spinal cord on day 20 of development (Experimental) Shows meningomyelocele, central dorsal splitting of the spinal cord, close approximation of the epimysium of the dorsal cervical muscle to the spinal cord and imperfect development of the dorsal parts of the vertebra and meninges.
Discussion

The present study focussed on the structural changes of the spinal cord in the developing foetus/pup of diabetic mother rather than gross malformations. This was based on several considerations. Currently, there are enough data showing that diabetic milieu causes gross malformations such as hydrocephalus, shortened hind legs and sacral agenesis (Becerra et al., 1990). However, no systemic study is found in the literatures about the structural changes of the spinal cord although early developmental changes in the neural tube have been studied by several investigators (Sadler, 1980; Cole and Transler, 1980; Horton and Sadler, 1983). In this study, asymmetry, dorsal horn defect, spinal cord protrusion, funiculus defects, enlarged central canal and underdevelopment (hypoplasia) of the spinal cord were observed. The present study investigating the structural abnormalities in the spinal cord during foetal development in diabetic mother is the first of its kind as verified by a vast review of literatures.

This study used streptozotocin-induced diabetic mouse mother, which produced defects in the foetus’s/pup’s spinal cord. It is unlikely that in the present study streptozotocin itself exerted any teratogenic effects since the drug was administered at least 1 week before the conception. Furthermore, studies have shown that radioactively labeled streptozotocin was completely eliminated from the body of the rat 6 h after intravenous injection (Karunanayake et al., 1979) and it does not affect the cleavage of rat morulae and blastocysts or neural tube folding of rat embryos in organ culture (Deuchar, 1979).

Since all the defects observed are not found in the same animal and some of the defects are expressed in different ways in different animals, it is reasonable to assume that the manifestation of functional behaviour of the animal might be different in different animals based on the type of defects present within a particular animal.

In addition, some of the defects are found in certain stages (such as on GD 14, GD 16, GD 18 or GD 20) and not in other stages. This must be considered as accidental occurrence since this study consisted of a few animals. However, in such circumstances, it may be interpreted that such isolated defects can be found at any other stages as well.

Implication of Functional Deficits in the Foetus/Pup Based on the Spinal Cord Defects Observed in the Present Study

Funiculus defects

Seventy-four foetuses/pups (38.9%) exhibited ventral, lateral or dorsal funiculus defects. The funiculus was eroded, shrunken and was obvious at almost all levels of the spinal cord in all the gestational days. Defects at any part of the funiculus will affect the sensory, motor and autonomic systems of the body.

A. Ventral funiculus defect

Motor deficits

Four major motor tracts (descending) should have been affected in the ventral funiculus defect: - vestibulospinal tract, reticulospinal tract, tectospinal tract and medial longitudinal fasciculus. The vestibulospinal tract, comprising lateral and medial vestibulospinal tract,
originates from vestibular nuclei situated in the pons and medulla and receives input from the labyrinthine system by way of the vestibular nerve and from the cerebellum. The prominent lateral vestibulospinal tract descends ipsilaterally in the ventral portion of the spinal cord until the lumbar level. It targets for the lower motor neurones and spinal interneurones associated with the innervation of the axial and proximal limb musculature, especially the extensor muscles (Burt, 1993). In general, lateral vestibulospinal tract excites lower limb extensor, upper limb flexors and axial extensors. Thus, a defect in this tract will lead to ipsilateral loss of control over these muscles. A defect of the medial vestibulospinal tract, which descends bilaterally until the cervical area, will cause a loss of regulatory control of the position of the head, neck and trunk regions in response to stimulation of the semicircular canal (Nieuwenhuys et al., 1998).

Originating from the reticular formation, the reticulospinal tract is important for both motor and autonomic functions (Crossman and Neary, 1998). A loss of this tract will result in the weakness of the flexors of lower limb, extensors of the upper limb and axial flexors, complementing the action of the reticulospinal tract. Furthermore, the postural adjustment and head movement will be affected if both vestibulospinal tract and reticulospinal tract are severed (Benarroch et al., 1999).

The tectospinal tract arises from the superior colliculi of the midbrain. Damage of this tract could disable or slow the head-turning in response to sudden visual or auditory stimuli (Nieuwenhuys et al., 1998). Similarly, the affected medial longitudinal fasciculus would lead to incoordination of head and eye movements. These two tracts descend only until the cervical region.

**Autonomic deficits**

Since reticulospinal tract has fibres connecting respiratory and circulatory systems, this might lead to lung and heart problems. Apnoea, asphyxia and hypertrophic cardiomyopathy were reported in children born to diabetic mothers (Krautzig et al., 1999; Sarici et al., 2001). On top of that, many of the newborn deaths reported in the literatures (Hawthorne et al., 1997; Boo, 1992) could have been due to respiratory failure or heart problems or both; possibly due to defects within the reticulospinal tract that arises from the vital centres of the medulla (e.g. respiratory and cardiovascular centres) (Crossman and Neary, 1998).

**B. Defect of the ventrolateral part of lateral funiculus**

The present result revealed that the defect in the ventrolateral funiculus was prominent in the thoracic region of GD 14 and cervical cord of GD 16 and GD 20. In this situation, the sensory (spinothalamic and ventral spinocerebellar tracts), motor (vestibulospinal and reticulospinal tracts) and autonomic tracts will be affected.

**Sensory deficits**

The spinothalamic tract carries information about light touch and pressure (anterior spinothalamic tract) as well as pain and temperature (lateral spinothalamic tract). This tract is functionally heterogeneous and includes second-order axons of nociceptive-specific, low-
threshold mechanoreceptive and particularly wide dynamic range neurones (Benarroch et al., 1999). Since spinothalamic tract spans the whole length of the spinal cord, any underdevelopment might cause a corresponding loss of these sensations on the opposite side of the body. However, light touch and proprioceptive sensations are retained if the dorsal column is not affected. This is termed “dissociated sensory loss” (Benarroch et al., 1999).

The ventral spino cerebellar tract receives information from the muscle spindle, Golgi tendon organs, touch and pressure receptors and decussates before terminating in the vermis. It sends signals from the lower extremities and trunk. The clinical symptoms caused by disordered spino cerebellar tracts are similar to Friedreich’s ataxia (Crossman and Neary, 1998; Simon et al., 1999; Waxman, 2000). Friedreich’s ataxia is an autosomal recessive disorder that begins in childhood (Simon et al., 1999). It is a degenerative disorder that leads to degeneration of spino cerebellar tract, posterior columns and dorsal roots as well as depletion of the neurones in Clarke’s column that are the cells of origin of the dorsal spino cerebellar tract (Simon et al., 1999). This disorder manifests as gait ataxia, weakness of the legs and intention tremor.

**Motor deficits**

Disturbance of both vestibulospinal and reticulospinal tracts might cause similar motor dysfunction as described earlier.

**Autonomic deficits**

Autonomic tracts are also involved when the ventrolateral funiculus is affected. Disturbance of the circulatory and respiratory systems occurs since reticulospinal tract is involved. Visceral sensation is carried by spinal visceral afferents that terminate in the spinal cord through paravertbral ganglia via the sympathetic and sacral parasympathetic trunks. Since spinothalamic and spino reticular tracts are involved in transmitting visceral pain, thus the pain could not be translated and adaptive, affective, autonomic and neuroendocrine responses are unable to be initiated (Benarroch et al., 1999).

**C. Defect of the dorsolateral part of lateral funiculus**

Underdeveloped dorsolateral funiculus is accompanied by corresponding dysfunction of the dorsal spinocerebellar and rubrospinal tracts.

**Sensory deficits**

The dorsal spinocerebellar tract produced the similar effects as described in the ventral spinocerebellar tract.

**Motor deficits**

In human, the rubrospinal tract does not extend below cervical levels and may be of little clinical significance (Burt, 1993). Both in human and rodents, this tract is responsible for precise and well-controlled movements (Nieuwenhuys et al., 1998).
D. Dorsal funiculus defect

Sensory deficits

Obvious improper development of the dorsal column was seen in GD 18 and GD20. The dorsal column tracts, which are a part of the medial lemniscal system, convey well-localised sensations of fine touch, vibration, two-point discrimination and proprioception from the muscles and joints. The fasciculus gracilis courses next to the posteromedian septum and carries input from the lower half of the body. Information of the upper half of the body is conveyed by the fasciculus cuneatus, which lies between the fasciculus gracilis and dorsal grey horn. Since both tracts ascend ipsilaterally and do not decussate until in the medulla oblongata region, a defect on one side of the spinal cord will cause ipsilateral deficits of the same side of the body (Burt, 1993). The most affected sensory functions are the two-point discrimination, stereognosis and graphesthesia. Other effects include unsteady gait (sensory ataxia), weakness and spasticity of limbs and poor ability to detect repeated stimuli and gradation of pressure stimuli (Benarroch et al., 1999).

Motor deficits

The corticospinal tract, in contrast to the human, is located in the dorsal funiculus of rodents (Kamiguchi et al., 1998). From the sensorimotor cortex, the descending axons pass through the ventrally located pyramids, cross the midline, forming the pyramidal decussation and turn dorsally. Then, they pass in the contralateral dorsal column of the spinal cord (Kamiguchi et al., 1998). Generally, the eroded or split dorsal column may have contributed to incoordination or loss of voluntary, discrete, skilled movements (Crossman and Neary, 1998).

Autonomic deficits

Autonomic functions such as micturation, defecation and gastric distension may be suppressed as these information are carried by the fibres in the dorsal column (Burt, 1993).

Dorsal horn defects

The structural changes observed in the dorsal horn include shortening, disorganization and erosion. These defects were obvious and account for the most defects in foetuses/pups of GD 16 (n=23), GD 18 (n=21) and GD 20 (n=26). Since dorsal horn is the site for primary sensory fibres termination (including all ascending tracts), loss or suppression of all kinds of sensory modulation should occur (Burt, 1993; Simon et al., 1999). When this happens, relay of sensory information to higher centres (e.g. thalamus, cerebellum and brain stem) is interrupted, thus cannot be interpreted and initiation of motor activity is refrained. This defect may account for diplegia, hemiplegia, paraplegia and quadriplegia as reported in children born to diabetic mothers (Fluge, 1975; Harlow et al., 1995; Stenninger et al., 1998) since the sensory loss generally affects the motor functions of related organ systems, relative to the severity of the defects.

Underdevelopment/hypoplasia

Hypoplasia of the spinal cord was observed in foetus of gestational day 16 (n=8), 18 (n=11) and
20 (n=6) and was common in the lumbar region as noted by Noden and Lahunta (1985). Feature of hypoplasia is indicated by large gap located either posteriorly or peripherally compared to the normal, thus showing that there has been a reduced growth. Clinical implications might be little since the grey and white matter seems to have developed normally. However, slight functional deficits might occur due to reduced amount of neurones depending on the severity of the defect. The reason behind the underdevelopment of the spinal cord is unknown. It could be due to increased apoptosis of neurones at the early stage of development or reduced mitotic activity or both and this is not supplemented by growth of these cells.

Spinal cord protrusion

Both ventral and posterior protrusions of the spinal cord were noticeable. Meningomyelocele was the most significant finding occurring at the cervical segments of the spinal cord. The segmental regions of this defect in the present investigation are in contrast to the available data where it is more frequent in the lumbar and sacral regions (Noden and Lahunta, 1985). This might be interpreted that this defect can occur at any segmental level. It is expected that this defect might affect the sensory, motor and autonomic functions of the whole body, depending on the level and severity of the defect.

Asymmetry

Asymmetry of the spinal cord was almost visible in all regions. Clinically, the impact of such defect is not significant as most fibres decussate and single neurological deficit cannot be easily interpreted. The patient or clinician may easily ignore minor degree of functional deficit on one side of the body. However, if the developmental defect is severe, the functional deficits should be easily identified.

Dilatation of central canal

Dilatation of the central canal was noticed in thoracic and lumbar regions of GD 16. This may due to delayed closing or imperfect closing of the neural tube as a result of delayed maturation or growth of neurones in the alar and basal plate during early development of the spinal cord. In addition, hydrocephalus might occur if this abnormality is severe in the cranial region.

Pathophysiology

Despite the recognition of the pathogenic potential of diabetic milieu on the spinal cord, the underlying mechanisms remain undetermined. The diabetic state has been reported as a rich source of teratogenic serum factors such as excess branched chain amino acids (α-ketoliscaproic acid) (Styrr, 1995), ketone bodies (Horton and Sadler, 1983; Buchanan et al., 1994) and glucose (Sadler, 1980; Suzuki et al. 1996; Moley et al., 1998a) itself. The mitochondria, being the source of energy to the cells, undergoes morphological changes when subjected to diabetic environment. Reports of mitochondrial derangement in neuroepithelium and vital organs (brain, heart and muscles) in embryos exposed to diabetic condition (Horton and Sadler, 1983; Yand et al., 1995) may give an insight to the changes observed in this study. The ultrastructural
changes of high-amplitude mitochondrial swelling might be an indication of biochemical or metabolic alteration (Horton and Sadler, 1983), especially in the enzymes, which are responsible for ATP synthesis. In addition, the presence of β-hydroxybutyrate may inhibit glucose utilization in embryos exposed to this compound while not providing an alternative fuel source due to minimal activity of tricyclic acid-oxidative phosphorylation pathways (Horton and Sadler, 1983). In this condition, the neurones are unable to thrive and mature properly, thus leading to delayed growth, immaturity and defects.

The defects observed may be explained as if due to the deleterious effects of hyperglycaemia-induced apoptosis and delayed or reduced mitotic division of neurones. Maternal hyperglycaemia has been shown to cause down-regulation of embryonic glucose transporters GLUT 1, GLUT 2 and GLUT 3 at blastocyst level (Moley et al., 1998a). GLUT 1 and GLUT 3 are found in the brain and neurones (Murray et al., 1996) and are essential for glucose uptake. Reduction of GLUTs leads to reduction of glucose intake and thus the survival of these cells is at stake. Apoptosis occurs when proapoptotic protein BAX is expressed thus activating caspase, DNA fragmentation and morphological changes consistent with apoptosis (Moley et al., 1998b). Apoptosis at this level might manifest later in the pregnancy as malformation of the nervous system.

Pax-3 gene may be accountable for the defects observed in this study. Pax-3 gene expression is important for normal embryonic development and survival (Phelan et al., 1997). Pax-3 is believed as a crucial inhibitor of this primary apoptosis event, which delays this process until neural ridges have migrated and are ready to fuse (Chang and Loeken, 1999). Spina bifida and anencephaly were noted in foetus/pup born to diabetic mouse mothers in experiment conducted by Phelan et al. (1997). In situ hybridisation on these defective foetuses/pups revealed threefold reduction in the Pax-3 mRNA expression. The effect of diabetic serum on the Pax-3 gene is undeniably true as verified by available literatures (Phelan et al., 1997; Cal et al., 1998). The changes occurred in this study have a high possibility being influenced by this particular gene.

Recommendations

A longer duration of time is required in order for this study to produce significant results. With a longer period, scanning and transmission electron microscopy study can be performed to probe into the ultrastructural changes especially in the mitochondria and other major cellular components. Other fields of study (e.g. molecular genetics and neuro-immunology) can be carried out to explore the underlying pathophysiology that causes the defects shown in this study.

Further investigations on the postnatal development of the spinal cord could be undertaken in order to evaluate the compensatory or accentuation of the defects observed in prenatal stage as indicated in this study. This is essential to understand not only the mechanism of neuronal behaviour including degeneration and regeneration but also the neurological and neurobehavioural abnormalities associated with the defects. Thus, this would permit possible interpretation of the neurological and neurobehavioural deficits in children born with such defects such as mental retardation, paraplegia and quadriplegia.
This study revealed that maternal diabetes mellitus exerts a profound, detrimental effect on the structure of the spinal cord of the developing foetus. Among the changes were funiculus defects, dorsal horn defects, underdevelopment of the spinal cord, spinal cord protrusion, dilatation of the central canal and asymmetry. All these changes can only be attributed to the diabetic environment in utero. It is beyond doubt that these structural changes contribute to the neurological deficits such as sensory, motor and autonomic loss. However, what really is the mechanism that contributes to such defects remains an enigma. Genetic and molecular changes might be accountable for these defects. Further investigation should be done to reveal the underlying mechanisms.

This study, being the first of its kind, is important in 2 ways: (i) it adds new data to previous findings that hyperglycaemia not only affects the early stage of neural tube development but also the subsequent development of spinal cord and (ii) it gives strong evidence to the basic causes of the abnormal neurophysiological behaviours such as paraplegia and quadriplegia as indicated by previous findings.

Foetal malformations associated with maternal diabetes are appalling. Thus, early management of maternal diabetes, whether before, during or after pregnancy, is essential for proper health of the mother and foetus.

References

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