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

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The present study is aimed at observing structural changes in the spinal cord of developing fetus of diabetic mouse mother. Diabetes was induced in 55 female mice (ICR strain) by two intra-peritoneal injections of streptozotocin. Those with blood glucose level ≥200 mg dL^{-1} were considered diabetic. Another 45 female mice served as controls without any diabetes induction. All these female mice were mated with normal healthy adult male mice. Pregnancy was terminated on Gestational Day (GD) 14, 16, 18 and 20 (day of delivery). Fetuses were fixed in 10% formaldehyde and serial sections were used for light microscopic study. Several abnormal changes were observed in the spinal cord in many fetuses born to diabetic mothers. The abnormal structural changes include asymmetry of the two-halves of the spinal cord, various types of structural deviations in the white matter, shrunken and eroded dorsal horns, imperfect growth and protrusion of parts of the spinal cord into the vertebrae, irregularity and dilatations of the central canal etc. These changes were not present in control samples. The findings in the present investigation implicated that the central nervous system is subjected to structural alterations in the developing fetuses when exposed to diabetic milieu. The structural changes observed are attributed to possible functional and physiological deficits. This confirms the neurobehavioral and habituation disturbances in the offspring of diabetic mothers. These results are supportive of the earlier clinical observations.

Key words: Diabetic mother, fate of fetal spinal cord

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INTRODUCTION

Over the past several years, the outlook concerning fetus and infant of a diabetic mother has changed remarkably following the discovery of insulin in 1922. However, the diabetic pregnancy is unique because of the diversity of problems that can affect the embryo/fetus beginning with conception. Considerable efforts, money and time have been devoted to understanding the basic developmental biology from observing young embryo *in vitro* and/or *vivo*. Evidence indicates that rigid glucose control will minimize the incidence of anomalies incurred before 9 weeks of pregnancy. The long-term effects of maternal diabetes are as diverse as the pathogenic events during pregnancy. Perinatal morbidity and mortality remained very high. However, there is improved prognosis for the infant of the diabetic mother. Previous studies have suggested that maternal diabetes mellitus may cause lasting effects on the psycho-neurological development in the offspring. Diabetic pregnancy represents both a clinical and research challenge in terms of its detrimental effects on the fetus. 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). The escalating incidence and prevalence of diabetes mellitus have placed this debilitating disease as one of the major threats to the global public health.

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 on the effect of maternal diabetes on the fetus focused on the gross morphological malformations. Histopathological changes were observed 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 analyzing the cause of neurological abnormality is conducted.

The present study is aimed to observe the histological changes of the spinal cord in the developing foetus of diabetic mouse mother and to relate the possible functional deficits arising out of the changes observed in the spinal cord.

MATERIALS AND METHODS

A total of 124 adult mice (100 females and 24 males) weighing 25.0 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 normal 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 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® Blood Glucose Monitoring System, Medisense®, Abbott). Animals with glucose concentration ≥200 mg dL^{-1} (≥11.1 mmol 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.

Mating: The mice were assigned to cages (15×28×38 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.

Termination of pregnancy and 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 caesarian section. Full-term pups were taken after delivery and sacrificed with an overdose of diethyl ether. The number of foetuses and pups were recorded.

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 and 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 center (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 Haemotoxylin-Eosin.

Statistical analyses were done by using SPSS Data Processing Version 10.0.

RESULTS

Structure of the lumbar segments of the spinal cord in fetuses/pups born to control mothers (control)

Gestational day 14: The lumbar segments of the spinal cord appeared well developed and fully occupied the vertebral canal. However, the central canal was large and elongated. The cord was normal looking with bilateral symmetry. Prominent grey and white matters were observed.

Gestational day 16: The spinal cord showed bilateral symmetry and fully occupied the vertebral canal. The central canal was smaller and less elongated (Fig. 1).

Gestational day 18: The spinal cord was well developed as indicated by the well-defined organization of the grey and white matters. Overall, the cord was oval and had bilateral symmetry. The cord had fully occupied the vertebral canal, leaving little space in between the spinal cord and the vertebrae. The central canal was significantly smaller and less elongated.

Gestational day 20: The spinal cord was well-developed and the grey and white matters were proportional having prominent dorsal and ventral horns. The white matter in the dorsal, ventral and lateral funiculi appeared normal on all sides of the grey matter. The spinal cord showed bilateral symmetry and had fully occupied the vertebral canal. The central canal was normal (Fig. 2).

Histopathological changes (defects) in the lumbar segments of the spinal cord of the fetuses/pups born to diabetic mothers (experimental)

Gestational day 14: The dorsal funiculi of the spinal cord showed central splitting on its dorsal surface, the split parts having tilted to lateral sides. The central canal was elongated. The vertebral arches and processes were not well developed. The spinal cord was very close to the skin showing under-development of several structures. There was no skeletal and muscular development posterior to spinal cord (Fig. 3).

Gestational day 16: There was imperfect and irregular development of the vertebral body. The ventral funiculus and ventral grey horn showed protrusion onto an ill-defined space within the irregularly shaped and misshapen vertebral body. The spinal ganglion was also protruding into the vertebral body lying closely adherent to the spinal cord projection. The ventral grey horn was elongated and extended into this spinal cord projection. This projection of the spinal cord and the misplaced spinal ganglion were located on the left side. The right half of the spinal cord apparently looked normal (Fig. 4).

Gestational day 18: The spinal cord was under-developed leaving a large space around the cord lying within the vertebral canal. The vertebra and its processes were also highly misshapen and irregular. However, the central canal appeared normal (Fig. 5).

Gestational day 20: There was a large space lying dorsal to the spinal cord and remaining within the vertebral canal. Spinal cord apparently looked normal having normal looking central canal. However, the vertebral body

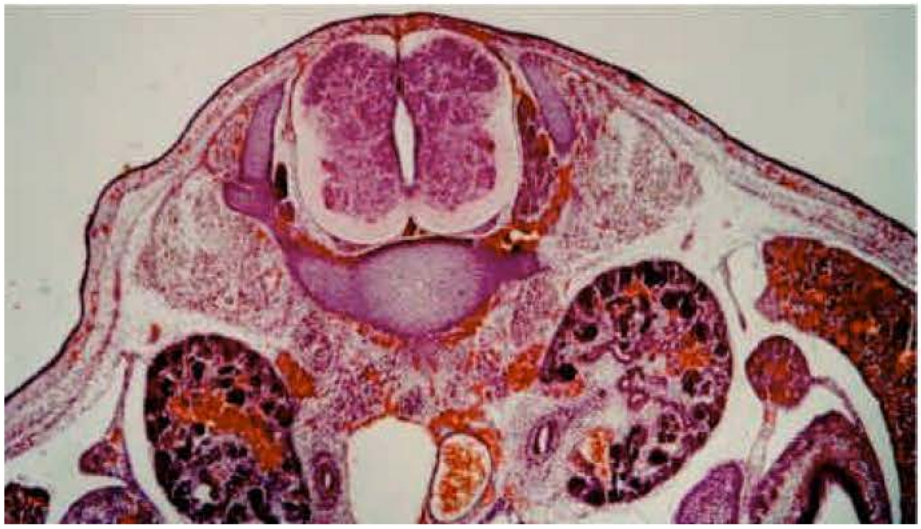

Fig.1: Gestational day 16, lumbar segment of spinal cord, control mother

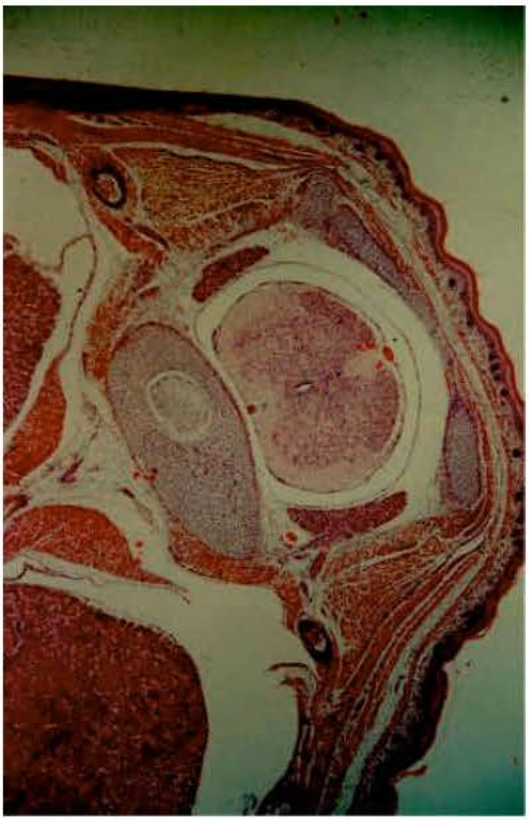

Fig. 2: Lumbar segment of the spinal cord, gestational day 20, control mother

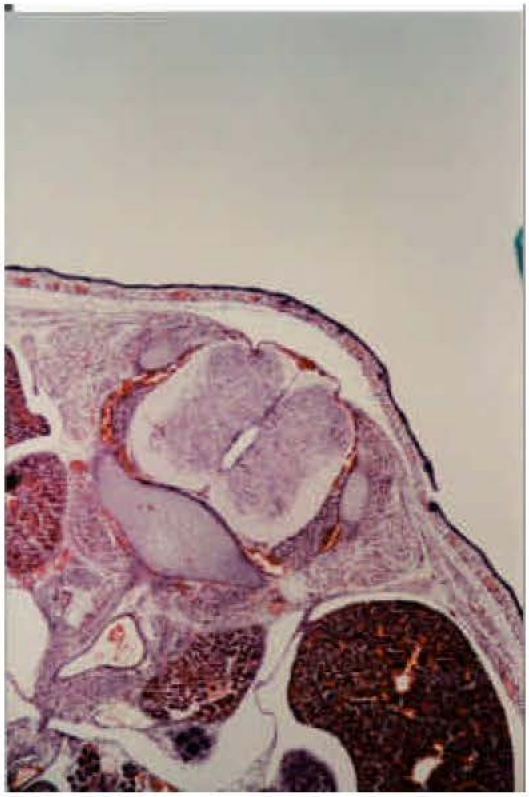

Fig. 3: Lumbar segments of the spinal cord, gestational day 14, diabetic mother (experimental)

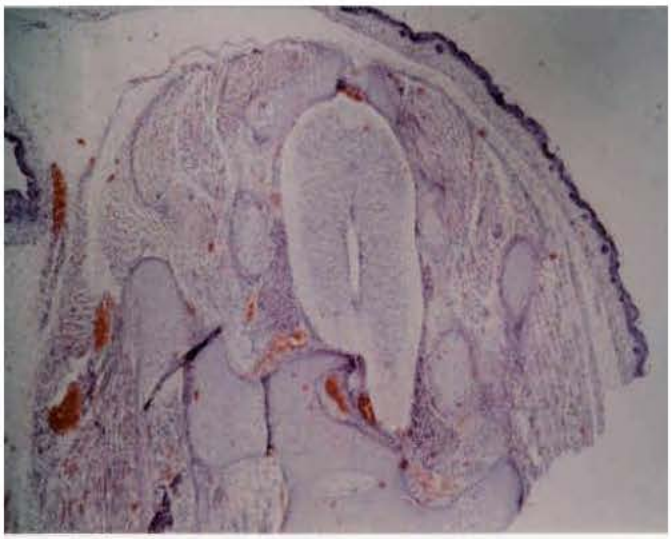

Fig. 4: Lumbar segment of the spinal cord, 16 days of gestation, diabetic mother (experimental)

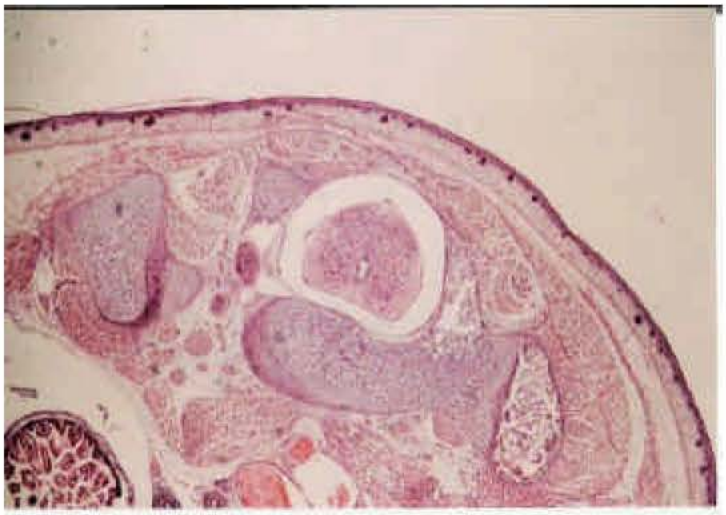

Fig. 5: Lumbar segment of the spinal cord, gestational day 18, diabetic mother (experimental)

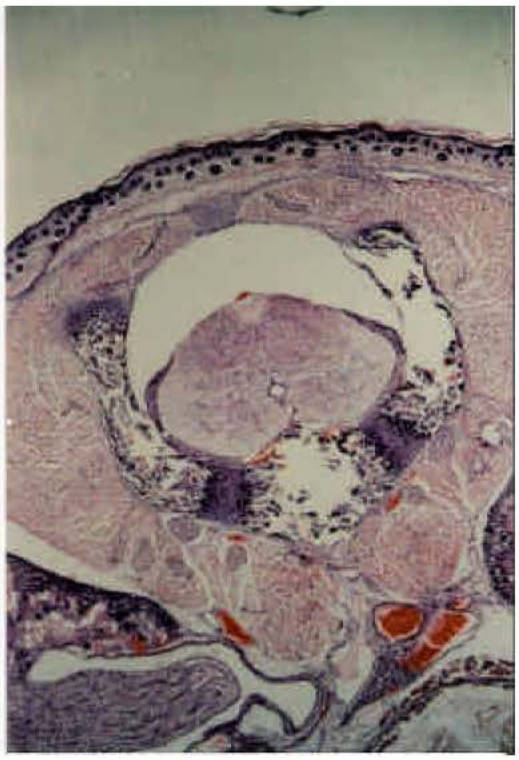

Fig. 6a: Lumbar segment of the spinal cord, gestational day 20, diabetic mother (experimental)

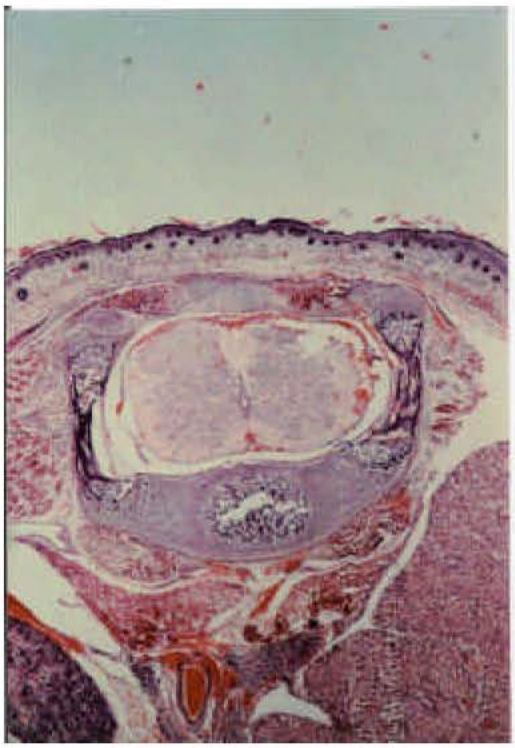

Fig. 6b: Lumbar segment of the spinal cord, gestational day 20, diabetic mother (experimental)

and its processes were greatly enlarged, thickened, rough looking and with developmental malformations (Fig. 6a).

In some fetuses the cord was irregular and blood clot was collected on its dorsal and left lateral aspects (Fig. 6b). The dorsal and left lateral funiculi had greatly damaged close to the blood clot. The erosion had extended onto a part of the dorsal column on both sides and dorsal grey horn on the left side. Ventral and right lateral aspects of the cord apparently looked normal. The blood clot (possibly hemorrhage) was visible in many places, especially on the ventral aspect of the vertebrae. Central canal was apparently normal looking. There was an irregular shaped space around the spinal cord that looked defective.

DISCUSSION

The structural changes of the spinal cord in the developing foetus/pup of diabetic mother are reflective of great functional and physiological deficits 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 labelled 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 defects observed in the spinal cord

Funiculus defects: In all seventy-four foetuses/pups (38.9%) exhibited ventral, lateral or dorsal funiculus defects. The funiculus was obviously eroded, shrunken 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.

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 coliculi 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 centers of the medulla (e.g., respiratory and cardiovascular centers) (Crossman and Neary, 1998).

Defects in 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 spinocerebellar 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 spinocerebellar tracts are similar to Friedreich's ataxia (Crossman and Neary, 1998; Waxman, 2000). Friedreich's ataxia is an autosomal recessive disorder that begins in childhood (Crossman and Neary, 1998; Waxman, 2000). It is a degenerative disorder that leads to degeneration of spinocerebellar 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 spinocerebellar tract (Crossman and Neary, 1998; Waxman, 2000). 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 paravertebral 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).

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).

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-localized 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). When this happens, relay of sensory information to higher centers (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). Hypoplasia is indicated by the large gap located either on the posterior or peripheral aspects compared to the normal, thus indicating a reduced development and 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. 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 because such spinal cord protrusions have been observed in other segments of the spinal cord as well (Pillay, 2003, 2004). 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-ketoisocaproic acid) (Styrud, 1995), ketone bodies (Horton and Sadler, 1983; Buchanan *et al*., 1994) and glucose (Sadler, 1980; Suzuki *et al*., 1996; Moley *et al*., 1998) 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; Yang *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*., 1998). 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*., 1998). 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; Cai *et al*., 1998). The changes occurred in this study have a high possibility being influenced by this particular gene.

The present study is important in two 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 clinical 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.

This study has 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.

Further investigations on the postnatal development of the spinal cord could be undertaken in order to evaluate the compensatory mechanism or accentuation of the defects observed in prenatal stage as observed in the present 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.

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