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

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The present experimental investigation was aimed to observe structural changes in thoracic segments of the spinal cord in developing fetus 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^{-1} were considered diabetic and mated with adult male mice. The other 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 microscopic observation demonstrated asymmetry of the two-halves of the spinal cord at thoracic segments of the spinal cord. Shrunken or eroded dorsal funiculi and dorsal horn represented the most frequent defect in the foetus/pup of GD 16 (23 in number), GD 18 (21 in number) and GD 20 (26 in number). In addition, the white and grey matters on the lateral and dorsal sides are much reduced in size or disappeared. Imperfect growth and protrusion of spinal cord were also observed. In some cases, a portion of the spinal cord has protruded beyond the vertebral laminae showing a condition called meningomyelocele. Irregularity and dilatation of the central canal were also noticed. These changes were not present in the control samples. The findings in the present investigation 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 clinical studies 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

Congenital malformations are one of the leading causes of perinatal mortality among children of diabetic mothers[1]. 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[2]. 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 report of the Second National Health and Morbidity Survey was found to be 8.2% of general population and is increasing by the tick of time. Generally, the incidence of congenital malformations is two- to five-fold higher compared to non-diabetic pregnancy[3,4]. 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[5]. 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[6-10]. Most of the previous studies that were done on the effect of maternal diabetes on the foetus focussed more on the gross morphological malformations. Histopathological changes were observed only on the early stage of neural tube development[11,12] but not on the later stages which results in the spinal cord and brain. Histopathological changes were observed in the neural folds of exencephalic embryos exposed to high glucose level and β-hydroxybutyrate[11,12]. 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[12]. This striking histological feature was actually highly amplituded-swelled mitochondria when observed under electron microscope. No particular study aiming to evaluate and analysing the cause of neurological abnormality is conducted.

The present investigation was conducted:

i) to observe the histological changes of the spinal cord in the developing foetus of diabetic mouse mother.
ii) 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 to 30 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 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, estrous cycle determination and mating: 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.

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.

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 and processing for light microscopy: 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 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 Haemotoxylin-Eosin.

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

RESULTS

Diabetic female mice parameters: In the control female mice, 20 out of 45 were confirmed pregnant.

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 of streptozotocin. Immediate deaths might be due to overdosage of anaesthesia or internal haemorrhage whereas deaths after a few days might be caused by diabetic ketoacidosis. Out of 31 streptozotocin-induced diabetic female mice, only 16 were pregnant successfully (Table 1).

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. Although the maternal weight gain/foetus during gestational day 14 and 16 for control was higher than that in the diabetic mothers, it did not prove to be significant.

Table 1: Maternal parameters in normal and diabetic mouse mothers

Group control	No of mothers	Maternal weight gain	No of fetuses/pups
GD 14	5	1.25	64
GD 16	4	1.41	52
GD 18	5	1.75	65
GD 20	6	2.12	73
Total	20		254
Diabetic			
GD 14	4	1.26	43
GD 16	4	1.78	47
GD18	4	1.46	48
GD20	4	1.30	52
Total	16		190

Maternal blood glucose level parameters and structural changes in fetal spinal cord: Control mothers' blood glucose level ranged from 5.0 to 10.5 mmol L^{-1}. However, the range of treated female mice blood glucose level was 11.5 to 25.4 mmol L^{-1}, thus confirming that they were diabetic (≥11.1 mmol L^{-1}).

Light microscopical observations on the spinal cord of the developing foetuses/pups of diabetic mothers revealed 5 major groups of structural changes, namely asymmetry of spinal cord, dorsal horn defects, funiculus defects, spinal cord protrusion and underdevelopment of the spinal cord. The proportion and seriousness of these defects varied in different animals and 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, there were mainly two structural changes, namely asymmetry (n=15) and, funiculus and dorsal horn defects showing the highest incidents with 21 foetuses. Overall, underdevelopment of the spinal cord constituted the least changes for all the gestational days.

Structure of the thoracic segments of the spinal cord in fetuses / pups born to control mothers (normal structure)
Gestational day 14: The spinal cord at thoracic regions appeared well developed with complete fusion of the neural folds and fully occupied the vertebral canal. However, the central canal was large and elongated. The cord was almost oval with bilateral symmetry. Grey and white matters were prominent and clearly distinct (Fig. 1).

Gestational day 16: The most striking differential feature was the smaller and less elongated central canal. The spinal cord showed bilateral symmetry and fully occupied the vertebral canal (Fig. 2).

Gestational day 18: The spinal cord was well developed as indicated by the defined organisation of the grey and

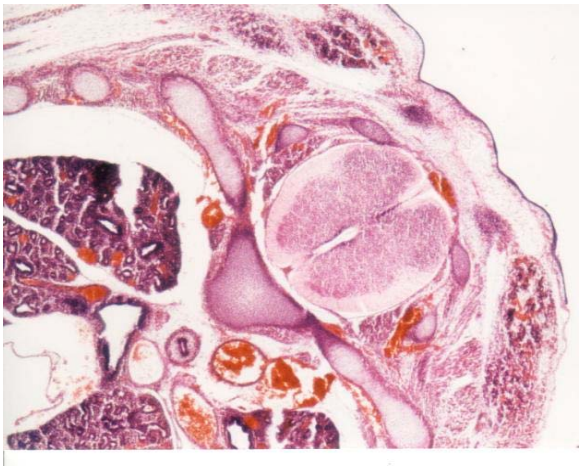

Fig. 1: Normal structure of the thoracic segments of the spinal cord on day 14 of development (Control)

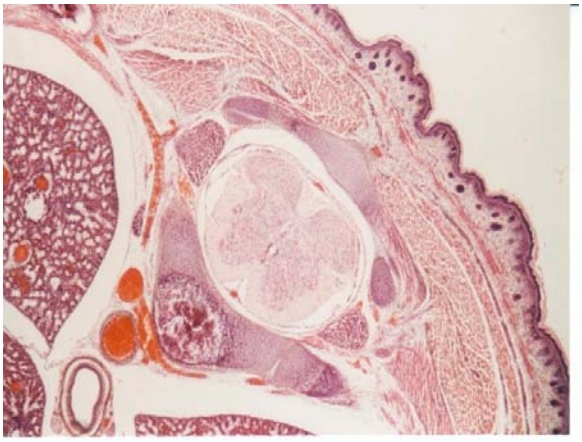

Fig. 4: Normal structure of the thoracic segments of the spinal cord on day 20 of development (Control)

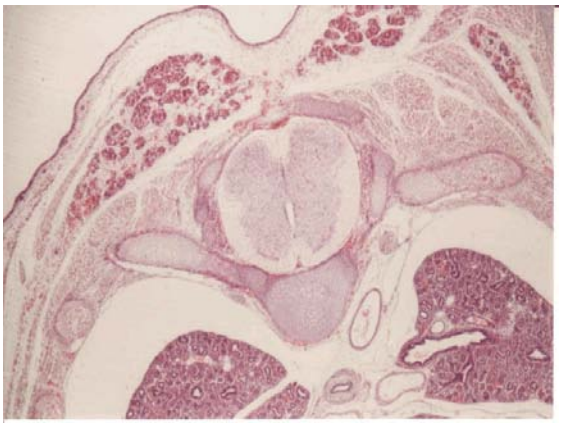

Fig. 2: Normal structure of the thoracic segments of the spinal cord on day 16 of development (Control)

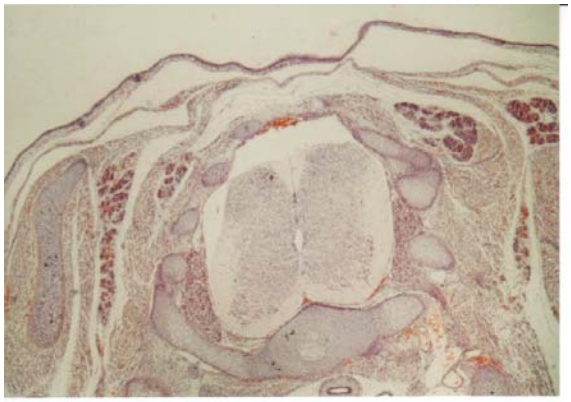

Fig. 5: Structure of thoracic segments of the spinal cord on day 14 of development (Experimental: pups of diabetic mothers)

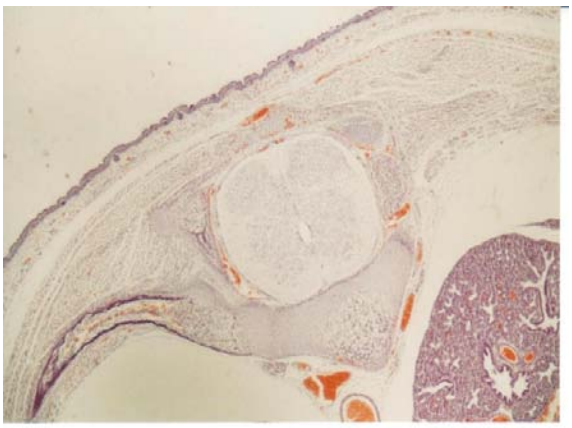

Fig. 3: Normal structure of the thoracic segments of the spinal cord on day 18 of development (Control)

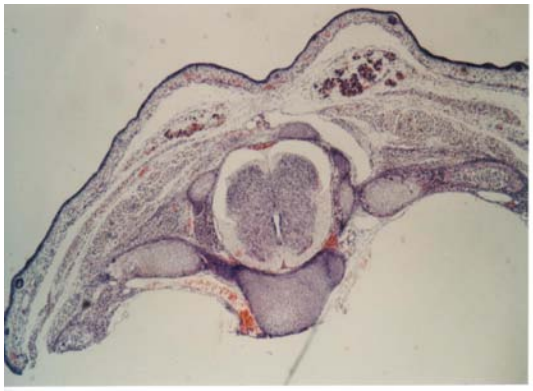

Fig. 6: Structure of the thoracic segments of the spinal cord on day 16 of development (Experimental: pups of diabetic mothers)

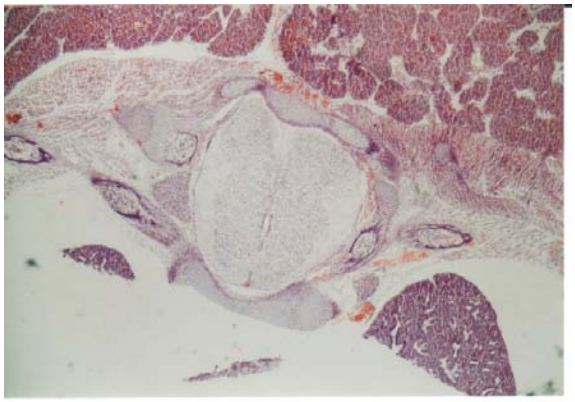

Fig. 7: Structure of the thoracic segments of the spinal cord on day 18 of development (Experimental: pups of diabetic mothers)

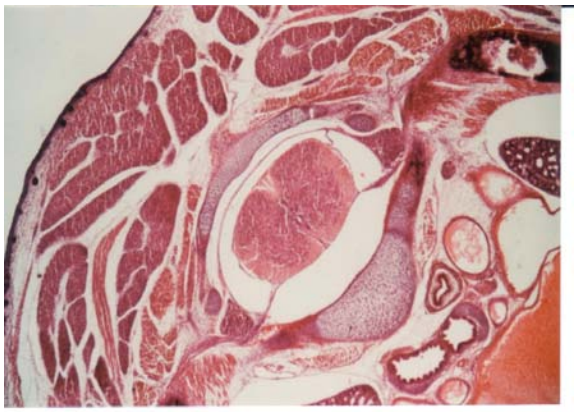

Fig. 8: Structure of the thoracic segments of the spinal cord on day 18 of development (Experimental: pups of diabetic mothers)

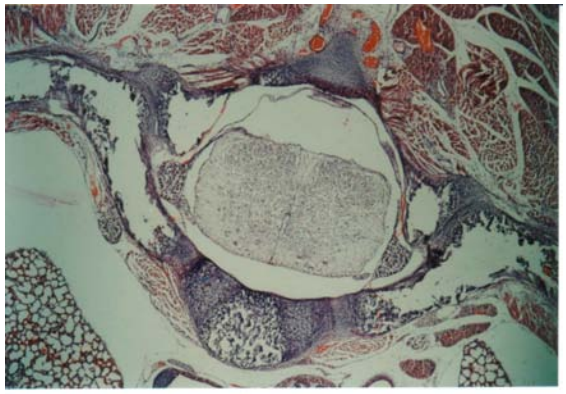

Fig. 9: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

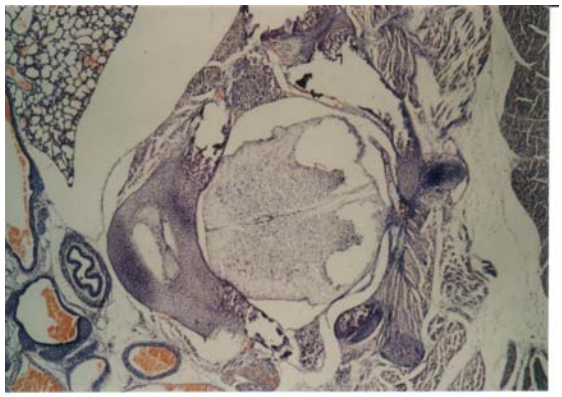

Fig. 10: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

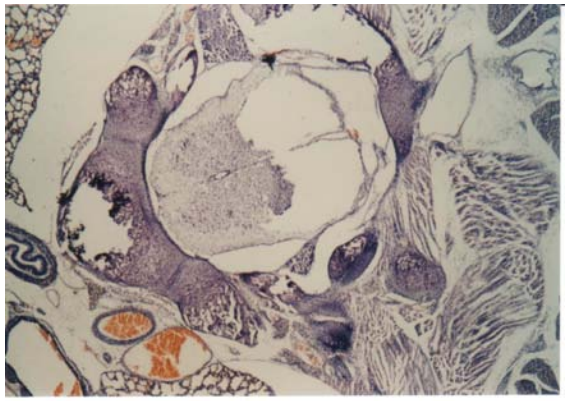

Fig. 11: Structure of thoracic segments of the spinal cord on day 20 of development (Experimental: pups of diabetic mothers)

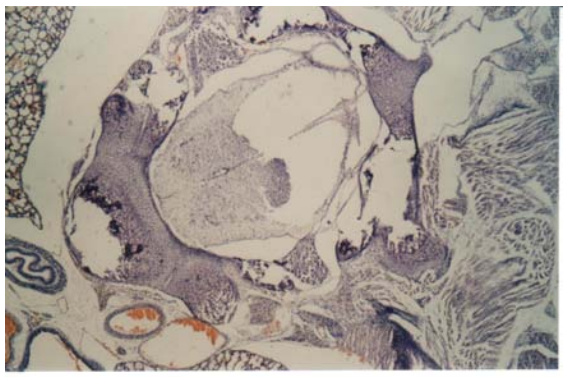

Fig. 12: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

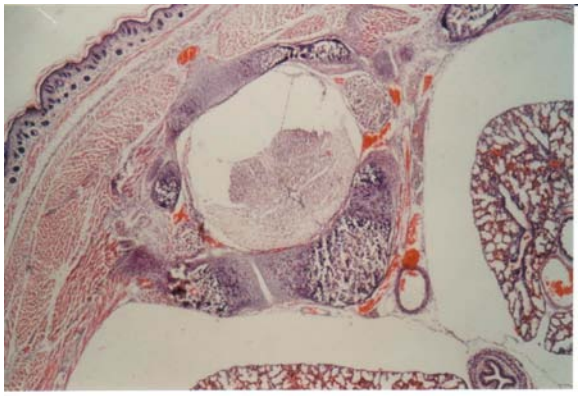

Fig. 13a: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

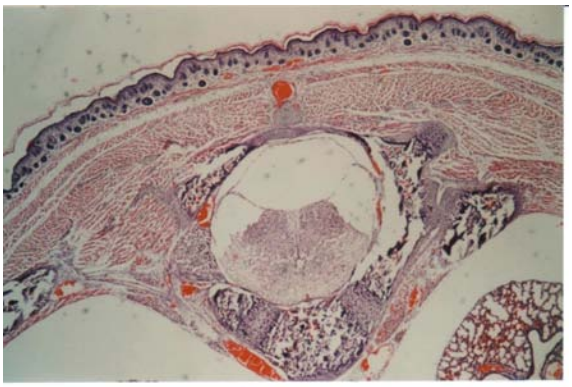

Fig. 13b: Structure of the thoracic segments of the cord on day 20 of development (Experimental: fetuses/pups of diabetic mother)

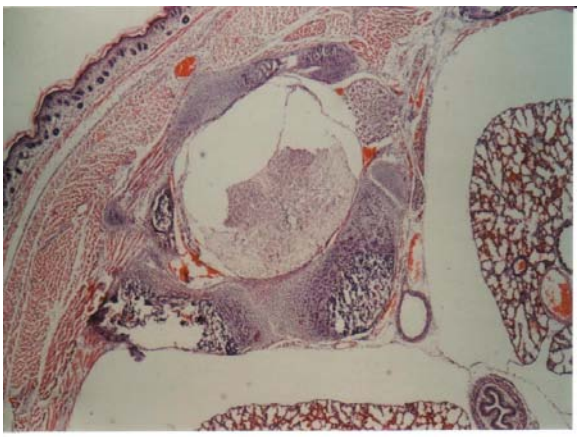

Fig. 14: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mother)

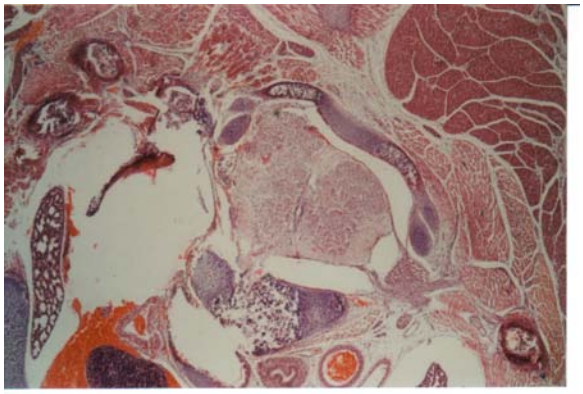

Fig. 15: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

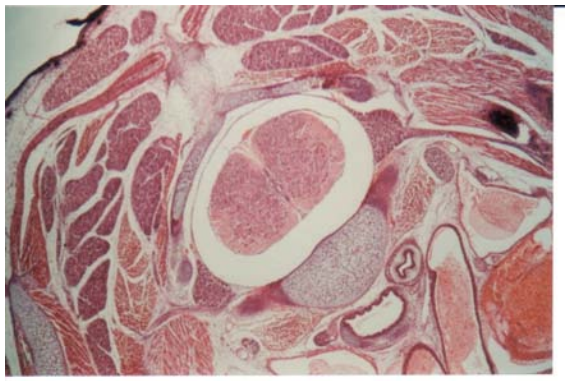

Fig. 16: Structure of the thoracic segments of the spinal cord on day 20 of development (Experimental: fetuses/pups of diabetic mothers)

white matters. The central canal was significantly smaller and less elongated. Two halves of the cord was symmetrical and the overall shape of the cord was oval. The cord has fully occupied the vertebral canal, leaving little space in between the spinal cord and the vertebrae (Fig. 3).

Gestational day 20: The spinal cord was fully developed. The grey and white matters were arranged properly with distinct features. Prominent dorsal and ventral horns were surrounded by well-defined ventral, lateral and dorsal funiculi. As noted in the previous gestational age (E18), the spinal cord showed bilateral symmetry and had fully occupied the vertebral canal (Fig. 4).

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

Gestational day 14: The dorsal portion of the thoracic

segment of the spinal cord was under-developed having a larger cavity visible within the vertebral canal. The dorsal funiculus, dorsolateral portion of the lateral funiculus including a small part of the dorsal horn were eroded. In some areas, the development of the vertebral laminae was not complete (Fig. 5).

Gestational day 16: The dorsal funiculus and major portion of the lateral funiculus including a large portion of the dorsal horn were eroded and disappeared. In some fetuses, the central canal was large and elongated. Other structures looked normal and intact (Fig. 6).

Gestational day 18: The posterior horn and posterior funiculus of the thoracic cord (Fig. 7) had protruded in a few animals. Profound asymmetry of the two halves of the cord was observed. The central canal and the remaining parts of the cord were apparently normal. The lateral funiculi were also well-developed and normal.

In some animals (Fig. 8) there was overall under development where the spinal cord diameter was small while the vertebral canal apparently had normal development. This left a larger space around the spinal cord within the normal-looking meningeal layers. The dorsal root ganglia and the spinal nerve rootlets were also apparently normal. The vertebral body and laminae were also normal.

Gestational day 20: In some animals (Fig. 9) the dorsal funiculi and a portion of the dorsal horn were greatly eroded. The cord was under-developed having large spaces between the spinal cord and meningeal layers.

The dorsal funiculi and dorsal horns on both sides of the cord showed a great deal of erosion in varying degrees. In some animals, there was a shrinkage or erosion of the lateral funiculi on both sides of the cord. There were ill-defined vertebral laminae and meningeal layers. A total disorganization of the cord and adjacent structures were clearly visible. However, ventral portion of the cord was less affected. In some animals (Fig. 10, 11, 12, 13 and 14) the erosion and other defects of the cord were greatly pronounced. Lateral funiculi and adjacent grey matter were also eroded in varying severity.

In some animals (Fig. 15) a total disorganization and asymmetry of the two halves of the cord (including white matter in dorsal, lateral and ventral funiculi and grey matter), shrinkage of one or other half of the cord, protrusion of the cord ventrally into the vertebral body, under-development of the vertebrae, disorganization of the vertebrae were found. Underdevelopment of the cord resulted in large spaces in the vertebral canal between the spinal cord and meningeal layers.

In some animals (Fig. 16), there was overall under development the cord having large spaces between the cord and meningeal layers.

DISCUSSION

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. Currently, there are enough data showing that diabetic milieu causes gross malformations such as hydrocephalus, shortened hind legs and sacral agenesis[4]. Early developmental changes in the neural tube have been studied by several investigators[11-13]. However, no systemic study is found in the literatures about the structural changes of the spinal cord. The present study focussed on the structural changes of the spinal cord in the developing foetus/pup of diabetic mother rather than gross malformations. In this study, asymmetry, dorsal horn defect, spinal cord protrusion, funiculus defects, enlarged central canal and underdevelopment (hypoplasia) of the spinal cord were observed.

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[14] and it does not affect the cleavage of rat morulae and blastocysts or neural tube folding of rat embryos in organ culture[15].

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. It is, however, to interpret that such isolated defects can be found at any other stages as well.

Implication of structural defects observed in the present investigation on the functional deficits that might result in the fetuses/new born

Funiculus defects: Seventy-four foetuses/pups (38.9%) exhibited ventral, lateral or dorsal funiculus defects. The funiculus was eroded and shrunken and the defect 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.

Ventral funiculus defect

Motor deficits: Four major motor tracts (descending tracts) should have been affected in the ventral funiculus defect. They are vestibulospinal tract, reticulospinal tract, tectospinal tract and medial longitudinal fasciculus. The vestibulospinal tract, comprising lateral and medial vestibulospinal tracts, originates from the 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[16]. 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[17].

Originating from the reticular formation, the reticulospinal tract is important for both motor and autonomic functions[18]. 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[19].

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[17]. 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[20,21]. On top of that, many of the newborn deaths reported in the literatures[22,23] 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)[18].

Defect in the ventrolateral part of lateral funiculus: The present results revealed that the defect in the ventrolateral funiculus was also prominent in the thoracic region of GD 14, 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[19]. Since spinothalamic tract spans the whole length of the spinal cord and crossed at every level, any underdevelopment might cause a corresponding loss of these sensations on the opposite side of the body below the level of the lesion. However, light touch and proprioceptive sensations are retained if the dorsal column is not affected. This is termed "dissociated sensory loss"[19].

The ventral spinocerebellar tract receives information from the muscle spindle, Golgi tendon organs, touch and pressure receptors and decussates before terminating onto the cerebellar vermis. It sends signals from the lower extremities and trunk. The clinical symptoms caused by disordered spinocerebellar tracts are similar to Friedreich's ataxia[18,24]. Friedreich's ataxia is an autosomal recessive disorder that begins in childhood. It is a degenerative disorder that arise from degeneration of the 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. 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 paraverterbral ganglia via the sympathetic and sacral parasympathetic trunks . Since spinothalamic and spinoreticular tracts are involved in transmitting visceral pain and thus the pain could not be translated and adaptive, affective, autonomic and neuroendocrine responses are unable to be initiated[19].

Defect of the dorsolateral part of lateral funiculus: Underdeveloped, defective or eroded dorsolateral funiculus will display and is accompanied by a corresponding dysfunction of the dorsal spinocerebellar and rubrospinal tracts.

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

Motor deficits: In human, the rubrospinal tract does not descend below the cervical levels of the spinal cord and may be of little clinical significance[16]. Both in human and rodents, this tract is responsible for precise and well controlled movements[17].

Dorsal funiculus defect

Sensory deficits: Improper development of the dorsal column was clearly seen in GD 18 and GD20. The dorsal column tracts, which are in most mammals 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 these functions on the same side of the body[16]. 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[19]. However, in rodents (including rats and mousse) the dorsal funiculus also contains corticospinal tracts for motor control. Therefore, in these animals motor function of cortical control will also beat least partially affected.

Motor deficits: The corticospinal tract, in contrast to the human, is located in the dorsal funiculus of rodents (including rats and mice)[25]. From the sensorimotor cortex, the descending axons pass through the ventrally located pyramids in the medulla oblongata, cross the midline, forming the pyramidal decussation and turn dorsally. Then, they pass in the contralateral dorsal column of the spinal cord[25]. Generally, the eroded or split dorsal column may have contributed to incoordination or loss of voluntary, discrete, skilled movements[18].

Autonomic deficits: Autonomic functions such as micturition, defecation and gastric distension may be suppressed as these information are carried by the fibres in the dorsal column[16].

Dorsal horn defects: The structural changes observed in the dorsal horn include shortening, disorganisation 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[16]. 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[7,26,27] 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[28]. 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 (Fig. 11 and 12) was the most significant finding occurring at the cervical and thoracic regions. 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[28]. 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-ketoisocaproic acid)[29], ketone bodies[12,30] and glucose[11,31,32] 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[12,33] 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[12], especially in the enzymes, which are responsible for ATP synthesis. In addition, the presence of β-hydroxybutyrate may inhibit glucose utilisation in embryos exposed to this compound while not providing an alternative fuel source due to minimal activity of tricyclic acid-oxidative phosphorylation pathways[12]. In this condition, the neurones are unable to thrive and mature properly, thus leading to delayed growth, immaturity and defects.

The defects observed in this investigation 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[32]. GLUT 1 and GLUT 3 are found in the brain and neurones[34] 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[32]. 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[35]. *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[36]. Spina bifida and anencephaly were noted in foetus/pup born to diabetic mouse mothers in experiment conducted by Phelan *et al.*[36]. *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[35,37]. The changes occurred in this study have a high possibility being influenced by this particular gene.

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.

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