

Spina Bifida: Morphological Features, Molecular Regulations and Signal Pathways

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Abstract

The main aim of this article is to review the knowledge about the closure defect of the vertebral column calls spina bifida. The anatomic, embryologic and molecular biologic knowledge about this condition is reevaluated with checking the contentful dry bone collection. Determined spina bifida samples, types in any part of vertebral column reviewed under the light of increasing recent literature. The review also gives attention to the cervical, thoracic regional, anterior and partial closure defect possibilities and clinical conditions, which are not emphasized adequately and are thought as a separate case status but are severe or mild types of spina bifida. Most common sites, especially sacral region of spina bifida and present incidence in different nations assessed and compared with our dry bone series. Regional incidence, types and clinical conditions of spina bifida reviewed under the light of the recent embryologic and molecular biologic studies. This review will give tidy think and aspect of morphology and clinics of spina bifida throughout of the vertebral column.

Keywords: Spina bifida; Neural tube defects; Developmental disease; Embryology

Definition and Background of Spina Bifida

Spina bifida means split spine [1]. It occurs when the spinal column does not close all of the ways or spinal column has closing alterations during gestation [2]. In the USA, about 1500 babies are born with spina bifida or brain and spine defects every year. It makes this the most common permanently disabling birth defect [3]. Because of a developmental abnormality of the spinal cord in the first trimester of pregnancy, spina bifida happens [4]. Anatomy of the vertebra, vertebral canal, contents and surrounding tissue is important for understand of embryologic development of spina bifida. Spina bifida emerges as a result of the non-closure of the upper part of the neural tube during embryonal development. It is classified into two main groups: open and closed spina bifida. The reason underlying it is not clearly understood. It is thought to arise as a result of genetic and environmental factors. Various other reasons which are unknown today are also thought to be effective.

We checked literature of vertebral anomalies, malformations and clinical conditions including spina bifida. A number of clinical studies have been published in the literature on the closure defect in one part of the vertebral canal and many gross clinical cases of neonate have been reported. Life-incompatible types of spina bifida are frequently mentioned by many authors. In addition, many anatomical studies have been done on dry bone of the sacral canal closure defects. The review gives attention to the cervical, thoracic regional, anterior and partial closure defect possibilities and clinical conditions which are not much emphasized and are thought as a separate case status but are severe or mild types of spina bifida. Regional incidence, types and clinical conditions of spina bifida reviewed under the light of the recent embryologic and molecular biologic studies. This review will give ordinate think and aspect of morphology and clinics of spina bifida throughout of the vertebral column.

Classical Anatomy of Vertebral Column and Vertebral Canal

Vertebral anatomy

The vertebral column in adult consists from 33-34 typical and

atypical vertebrae. Typical vertebra has two main part, the body and the vertebral arch. The vertebral arch comprises pedicle of the vertebra and lamina. Fusion of left and right laminae forms the spinous process of the vertebra (Figures 1a and 2a). The vertebral foramen locates between body and vertebral arch [5]. First cervical vertebra atlas is an atypical vertebra, given name atlas. There is no vertebral body of the atlas and it has anterior and posterior arches [6]. Sacral and coccygeal atypical vertebrae synostosis to form sacrum and coccyx as a single bone in adult [5].

Vertebral canal

Consecutive alignment of vertebral foraminae form the vertebral canal. Anterior wall of vertebral canal consists of vertebral bodies, intervertebral discs and posterior longitudinal ligament (Figure 2a). The vertebral arches form lateral and posterior sides of the canal. There are openings of bony vertebral canal laterally and posteriorly. Intervertebral foraminae between consecutive two vertebrae are lateral exit of the canal. Posterolateral opening between consecutive vertebral arches are closed with ligament flava and interspinous ligaments (Figure 2a) [7].

Part of vertebral canal in sacrum calls sacral canal differs with some features from the rest of the vertebral canal. The posterior wall of sacral canal is completely closed via fusion arch of lamina and spinous processes of S1-S4 vertebrae and ligaments between arches. In the majority of society lamina of the fifth vertebra is agenized and

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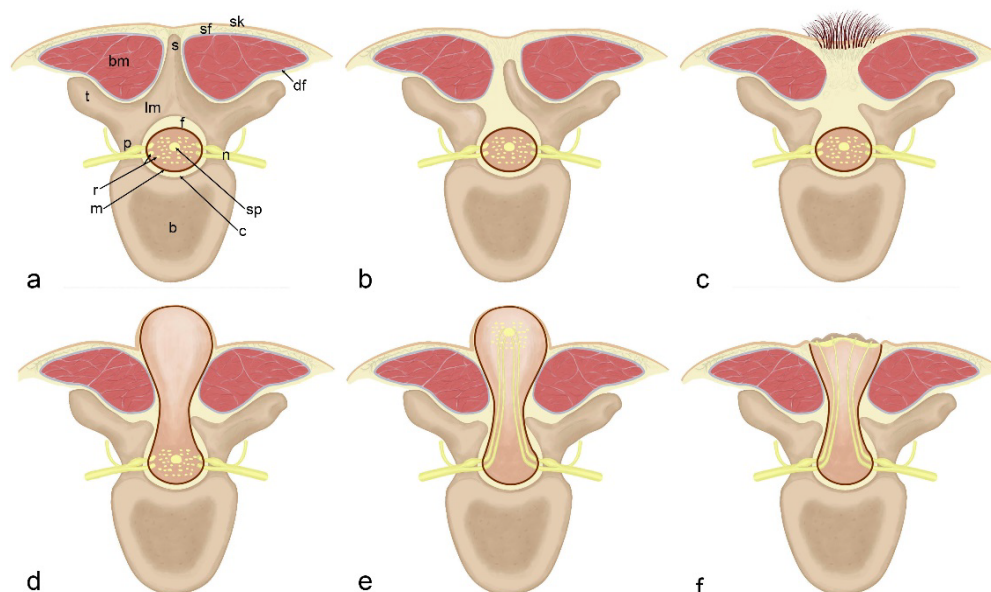


Figure 1: Normal anatomy and cross section of spina bifida patterns at lumbosacral region. a. Normal lumbosacral anatomy at lumbar 3 vertebra, b. Spina bifida occulta result of partial agenesis of posterior vertebral arch and little pit on the covering skin, c. Tuft of hair over complete defect of posterior arch in spina bifida occulta, d. Meningocele, e. Myelomeningocele and f. Myeloschisis (rachischisis). (Abbreviations: b, body of vertebra; bm, back muscles; c, vertebral canal; f, fibroadipous tissue in vertebral canal; lm, Lamina of vertebra; m, meninges; n, spinal nerve; p, pedicle of vertebra; r, roots of spinal nerve; sf, superficial fascia; df, deep fascia; sp, spine; s, spinous process; sk, skin; t, transvers process).

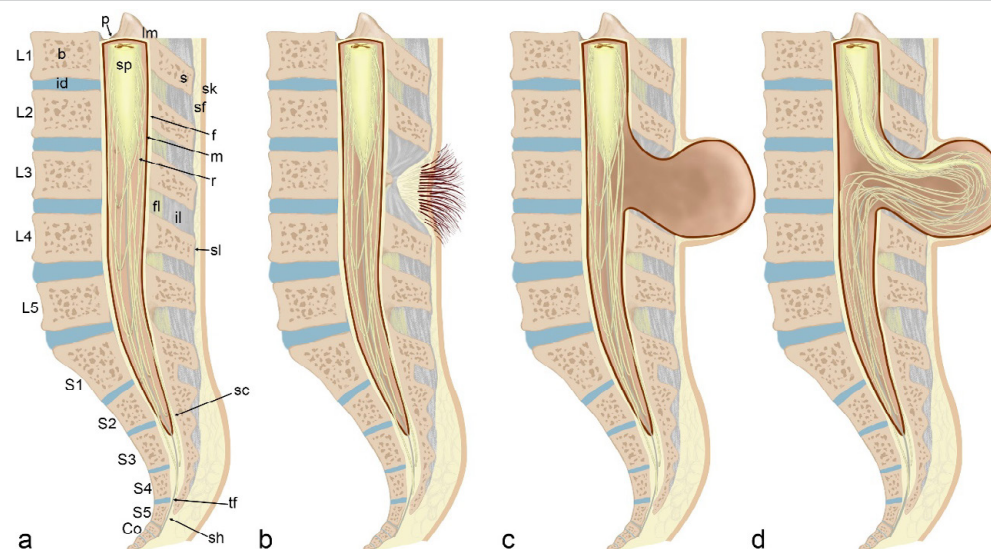


Figure 2: Lateral view of normal anatomy and spina bifida patterns. a. Normal anatomical pattern of lumbosacral region, b. Tuft of hair over complete defect of posterior arch in spina bifida occulta, c. Meningocele and d. Myelomeningocele. (Abbreviations: b, body of vertebra; Co, coccygeal vertebrae; f, fibroadipous tissue in vertebral canal; fl, ligamentum flava; id, intervertebral disc; il, interspinous ligament; lm, lamina of vertebra; m, meninges; p, pedicle of vertebra; r, roots of spinal nerve; L1, L2, L3, L4 and L5, number of lumbar vertebra; S1, S2, S3, S4 and S5, number of sacral vertebra; sc, sacral canal; sf, superficial fascia; sh, sacral hiatus; sk, skin; sl, supraspinous ligament; sp, spine; tf, terminal filum).

resulted in form of sacral hiatus (Figure 2a) [8]. Sacral canal presents four intervertebral foramina at lateral side of canal. The intervertebral foramina of sacrum divide and opens as an anterior (pelvic) and posterior (dorsal) sacral foramina which give exit to anterior and posterior roots of spinal nerve [7].

Contents of vertebral canal

Vertebral canal contains vessels, meninges, nerve tissue (spine, roots of spinal nerve) in fibroadipous tissue. Spine floats in subarachnoid cerebrospinal fluid and ends at the level of the L1 or L2

vertebra in adult. The lower tip of spinal cord (medullary cone) gives rise to terminal filum attaches to behind of first coccygeal vertebra through sacral hiatus (Figures 1a and 2a). Anterior and posterior nerve roots left from spine fuses to form spinal nerves exit vertebral canal form intervertebral foramen. Lower spinal roots elongate down to reach owns intervertebral foramen. The elongation of root forms horse tail shape calls cauda equina. Back muscles, such as erector spinae, covered with deep fascia (thoracolumbar fascia) leis lateral to spinous process. Fibroadipous superficial fascia and most outside skin surround vertebral column posteriorly (Figures 1a and 2a) [7].

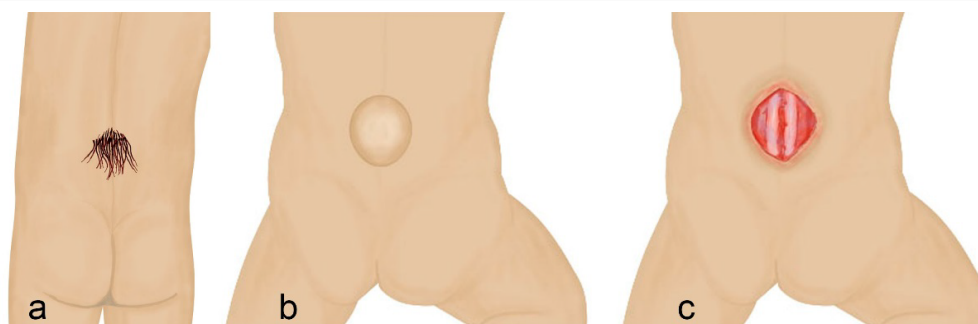


Figure 3: Posterior view of back in spina bifida a. Tuft of hair on the skin cover spina bifida occulta in child, b. Spina bifida aperta with meningocele or meningomyelocele in infant and c. Myeloschisis pattern of spina bifida aperta in infant.

Complete or partial non-fusion of laminae or defect in the vertebral arch results in the formation of spina bifida. In spina bifida, contents of vertebral canal have opening joints to surrounding tissues of canal [9-12].

Embryology and Pathogenesis of Spina Bifida

During embryonal development, sometimes the neural tube closure is not completed as a result of the lack of chorda dorsalis' (notochord) induction effect on the ectoderm or teratogenic effects. In parallel to the developmental disorder in the neural tube, developmental disorders can also be seen in the bone tissue, that is, the skull and the vertebra. In bone disorders, the upper part of the neural tube remains open. If this defect occurs throughout the whole nerve system, it is called "complete rachischisis"; if it occurs in a small specific region, it is named "partial rachischisis" [13-16].

The backbone and covering membranes cannot form and close properly within the first 4 weeks after the fetus is conceived. These membranes and backbone protect spinal cord. Forming and closing processes may result in an opening somewhere along the spine, with damage to the spinal cord and spinal nerves [6]. Defects generally seen in the spinal cord regions are called spina bifida (spinal dysraphism, spinal cord opening). If these anomalies emerge at the cephalic region, they are called anencephaly [14]. Spina bifida refers to a variety of disorders. This phenomenon occurs mainly in the lumbosacral region (L5-S1) and the medulla spinalis is covered by skin only and the open bone is not seen from outside. Sometimes, there may be a small amount of hair growth over this region. This anomaly which occurs in a single vertebra is called spina bifida occulta. It is mostly seen in L5 or S1. The prevalence of it in humans is 10%. It does not generally yield clinical symptoms [16]. The type which leads to a vertebral arc defect, like protrusions in the meninges or accompanying cystic sacs on the medulla spinalis is called spina aperta (cystica). If there are defects in more than one or two vertebrae, the membranes of the spinal cord (meninges including dura mater, arachnoid and pia mater) extend outside of this open area. This defect, which is covered by skin, is called meningocele. If along with meninges, the spinal cord also extends to the sacs, it is named as meningomyelocele [13-17]. Meningomyelocele is mostly accompanied by neurological symptoms [14]. In another type of spina bifida, when the defect is larger, the medulla spinalis and spinal nerves are in the open along with the meninges and the nerve tissue is dispersed to a larger area. Sometimes neural tissue proliferation may be observed [13,14]. This anomaly is called myeloschisis (myelocele or rachischisis) [13-15,17].

Types of Spina Bifida

Spinal bifida is a general term which refers to a variety of

phenomena, e.g., the absence of a minimal spinose protrusion, various vertebral anomalies, and pathologies of varying levels, which may be accompanied by the spinal cord and nerve root. In order to minimize such controversies and provide ease of diagnosis, it is considered to be appropriate to divide spina bifida into two main groups: Spina bifida occulta and spina bifida cystica (aperta).

Spina bifida aperta (meningocele, meningomyelocele, myeloschisis)

Meningocele, the sac-like structures containing the spinal cord meninges and the cerebrospinal fluid may rupture outward (Figures 1d, 2c and 3b). This type may cause minor disabilities, but usually there is not any nerve damage, but little [18].

Myelo-meningocele, is the most dramatic form of spina bifida. The vertebra and its membranes extend outward from the vertebra opening. It is covered with skin in some (myelo-meningocele) and remains open in some others (myelocele). Myelocele and myelo-meningocele are also called spina bifida manifesta (Figures 1e, 2c and 3b). It is known as open spina bifida. This type is the reason of moderate to severe disabilities [19].

Myeloschisis (rachischisis) is the severest form of the spina bifida aperta. Importantly, The neural tissue, called "placode", has no dermal or meningeal covering (Figures 1f and 3c). Complete closure of the neural folds fails to occur in this anomaly [20]. This is the most complicated form of spina bifida. In such cases, the affected area is open because the neural tube convolutions are not closed [17].

Spina bifida occulta

Spina bifida occulta, only one vertebral arc is not developed. It is covered with skin. There is hair growth in this region. Generally, the spinal cord and the nerves are normal (Figures 1b, 1c, 2b and 3a). When one looks from the outside, it is observed that some infants have a dimple, dark spot, hairy patch, or swelling at the affected area, but the spinal cord and the nerves generally are not damaged (Figures 1c, 2b and 3a). This type of spina bifida usually does not lead any disabilities. Spina bifida occulta may call hidden spina bifida, because it could not be discovered until the end of childhood or adulthood and sometimes all lifelong [21].

Occult Spinal Dysraphism (OSD), Infants with OSD have a dimple in their lower back (Figure 2b). But it does not mean that all babies with dimple have OSD. Hyperpigmented patches, red marks on the back and tufts of hair or small lumps are the other sights. In OSD, the spinal cord may not grow the normal way and this situation can cause serious problems as getting older [22].



Figure 4: Samples of spina bifida from dry bone collection of Ege University Faculty of Medicine, Department of Anatomy. a. Cervical spina bifida, posterior arch defect of atlas. b. Fusion defect of lumbar vertebra and c. Sacral spina bifida, complete dorsal wall agenesis of sacrum.

Developmental Embryology and Molecular Regulations of Spina Bifida

It is vital to know the molecular mechanisms of neural tube closure so as to gain insight into the embryological basis of NTDs, including spina bifida [2]. The process of primary neurulation is responsible for developing of the brain and spinal cord regions down to the S4-S5 levels. Embryologically, secondary neurulation takes initiative as the remnant of the cord. The neural tube develops from mesoderm cells [23]. These epithelial cells organize around lumen to form the caudal regions of the neural tube that then becomes continuous with the remnant of the tube formed by primary neurulation [4].

Posterior defect of atlas ranges from hypoplasia with cleft to total aplasia of posterior arch (Figure 4a) [6,24]. Some authors describe posterior defect of atlas as a spina bifida occulta of atlas [25,26]. Currarino et al. proposed a classification of defect of posterior arch of atlas [6]. Occult spina bifida of atlas may be misdiagnosed as a fracture [27]. Spina bifida aperta of atlas is a severe type [28]. Blaauw dissected the posterior arches of the cervical vertebrae of 30 fetuses died with myelo-meningocele in the lower thoracic, lumbar or sacral region [29]. There was posterior arch defect of 70% of fetuses and separate parts of posterior arch can connect each other by fibrous band [29]. Congenital defect of atlas associated with syndromes affecting craniovertebral junction including Down's syndrome, Arnold Chiari malformation, Klippel-Feil syndrome [26,30]

Atlas arch defects are found nearly 4% and most common defect has characterized by a small dorsal cleft (3.2% of all patients) [31]. Most of adult patients live with an occult spina bifida of atlas are asymptomatic. In symptomatic cases intermittent prickling, paresthesia, motor symptoms like hemiparesis or quadriparesis can present in episodic nature [25]. The defect of atlas also causes misdiagnose fracture or coexist with it. It may restrict neck movements and activities like strenuous sports [32].

The most frequent location of occurrence of spina bifida respectively is the lumbosacral region in 50% of cases, the thoracolumbar area in 35% of cases [33]. Vertebral cleft of thoracic or lumbar vertebra noticed

randomly in imaging (Figure 4b) Cleft in thoracic vertebrae can be concomitant part of multiple anomalies [34,35]. Totally of lumbar vertebral arch may absent and may be part of lumbosacral spina bifida [36]. Complete dorsal wall defect of sacrum has been reported many times (Figure 4c) [37]. Complete dorsal wall agenesis of sacrum differs from country to country. Also partial defect of sacrum as occult spina bifida at L5, S1 and S2 reported respectively 0.3%, 3.8% and 4.0% [38].

Because of the defective vertebrae attachment of back muscles changes and in elder age's occult spina bifida in lumbosacral region causes back pain [39-41]. However, patients have occult spina bifida with low back pain that lasted longer than 2 weeks did not differ from without spina bifida patient in terms of neurologic sign [42].

Occult lumbosacral spina bifida may be problem in epidural anesthesia and screw fixation in lumbosacral region [43]. In progressive ages with spina bifida occulta 13.7% of the patients have problem with the overactive bladder [44]. Anterior spina bifida more rare than posterior and fetuses can be diagnosed with magnetic resonance imaging [45]. In elder ages anterior meningocele of sacrum projected into the retro-peritoneum of pre-sacral space is reported during pregnancy. It can be asymptomatic even in old ages, but it can be complicated labor and delivery [46]. We didn't observe separation of sacral anterior surface in our dry bone series.

Central nervous system anomalies have the highest prevalence (34.7%) in congenital anomalies and spina bifida/meningocele were the most common (44%) central nervous system anomaly [47-49]. The mandatory the fortification of folic acid in preconceptional period reduced live births, stillbirths and terminations of pregnancy due to spina bifida [50]. The prevalence of spina bifida among children and adolescents (0-19 age groups) has been reported 3.1 cases per 10,000 [51]. People living with spina bifida have multiple deficiencies, such as physical insufficiency, incontinence, back pain and need multidirectional care. Family and public should be informed about care and behavior of person with spina bifida.

Discussion on the Current Investigations on Effective Molecules and Signal Pathways on Spina Bifida

As with most complex biological processes, there is the possibility

Abbreviation	Roles
DLHP	Neural plate folding hinge point
MHP	Neural plate folding hinge point
ZIC2	Regulates the kinetics of neurulation
HNF3	Required for notochord development
FOX a2	Direct transcriptional targets of Shh signalling
GLI1/2	Direct transcription altargets of Shh signalling
SHH	One of the main pathway and regulator
PTHC1	The gene has a role but not clearly understood
TNIP1	New potential associated gene

Table 1: Abbreviations and potential roles of different genes, proteins and molecules in the regulation of neural tube shaping during embryogenesis.

of functional redundancy in neurulation, the loss of a single gene product prevents the nerve tube from closing. Also, the cranial region is more susceptible to genetic predisposition than the spinal cord. It is an observation parallel to similar results when NTDs are produced with teratogenic agents [52]. In humans, anencephaly is less common than spina bifida and suggests head neurulations, which is probably stronger than it is in mice. Differential use of MHPs (medial hinge points) and DLHPs (dorsolateral hinge points) at different axial levels means a transition as the neurulation progresses, dorsolateral bending over the midline. DLHPs' appear is required for low backbone neural tube closure because mice lacking the ZIC2 transcription factor exhibit normal MHP but fail for DLHPs development and large spina bifida development [53-55]. Conversely, the formation of a defined midline bend is not necessary for closing. During the terminal period of spinal cord neurulation, the posterior neuropore does not have a defined MHP and are completely mediated via DLHPs. Indeed, embryos lacking notochord do not show MHP, but they form ectopic DLHPs throughout the entire neuroaxis and may complete the neural tube closure. Indeed, embryos lacking notochord do not show MHP, but they form ectopic DLHPs throughout the entire neuroaxis and may complete neural tube closure [56]. For this reason, spinal nerve tube closure continues with a presumed mechanism involving only DLHPs in the absence of the MHP. The lack of an MHP requirement in neural tube closure is supported by the fact that spinal NTDs do not appear in mouse embryos lacking sonic hedgehog (SHH), HNF3 (FOX A2), GLI2 or GLI1/2, all them fail to form a floor plate. In these targeted mouse mutants, the nerve tube is lumen circular, indicating that closure is mediated by a DLHP-dependent mechanism [2,57,58].

Beside these, there are also new studies that are enlightening the complex mechanism of spina bifida when the genetic cause of the relationship between GLI2 single nucleotide polymorphisms and spina bifida risk is investigated. The methylation status of the GLI2 promoter region between the spina bifida and control individuals and the area near the transcription initiation site were compared. In addition, the possible correlation between folate level, GLI2 methylation status, and spina bifida is also assessed. GLI2 protein expression was significantly reduced in spina bifida brain tissues; GLI2 hypermethylation was associated with high formation of spina bifida. IHC analysis of brain tissue revealed that GLI1 was expressed in spina bifida specimens at lower levels than control samples in spina bifida specimens and that PTH1 expression was higher in the spina bifida group, but no difference was significant. This indicates that in the brain tissue embryos with spina, the SHH pathway is suppressed. Data of Lu et al. come to a conclusion of epigenetic silencing of GLI2 may have an important role in the development of spina bifida during embryogenesis [59]. Cladia et al. suggested that TNIP1 is a new potential associated gene to spina bifida. But still its pathway needs to be investigated in human NTDs to confirm its role [60] (Table 1).

Previous case reports showed the importance of understanding embryonic development malformations [61-64]. A recent case report from Taiwan showed us that embryonic development defects combined with spina bifida may be fatal [65]. Here, they reported a rare case of parapagus diprosopus twins (12-weeks' gestation) associated with spina bifida. They observed two posterior fontanelles. They used 3D tomographic ultrasound imaging to investigate the fetal spine [65].

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