CONGENITAL ANOMALIES IN THE VERTEBRAL COLUMN ASSOCIATED WITH THORACOLUMBAR TRANSITIONAL VERTEBRAE

Anneli Merlé Du Plessis

Thesis presented in fulfilment of the requirements of the degree Master of Science at Stellenbosch University

Supervisor: Mrs L.M. Greyling

December 2017

DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication therefore by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2017

ABSTRACT

According to Byrd & Comiskey (2016), disrupted ossification during development results in abnormal skeletal development. A study conducted on congenital anomalies by Masnicová & Beňuš (2003), stipulated that most skeletal congenital defects are located in the vertebral column. The most common skeletal defects of the vertebral column are neural tube defects (NTD's), spondylolysis and cranial-caudal border shifts (Masnicová & Beňuš 2003). In reviewed literature, case studies have reported various congenital defects that are simultaneously present within the vertebral column of an individual. There is, however, a lack of evidence to substantiate whether the mutually inclusive observations resulted by chance, or whether an association between the defects is present. The aim of this study was to determine whether associations exist among random congenital defects in the vertebral column. The objective of this study was to identify and determine the frequency of random congenital defects from a subset of defects in the vertebral column. A selection of skeletal remains were taken (n=35) from a subset in the Kirsten Skeletal Collection at Stellenbosch University. The subset comprised specimens from the population (N=±1100) with congenital defects in the vertebral column that has a reviewed prevalence of 0.5/1000 worldwide. This study hypothesised that there is an association between random congenital defects that results from border shifts or disrupted neural arch formation. The congenital defects considered in the study included: lumbosacral transitional vertebrae (LSTV), thoracolumbar transitional vertebrae (TLTV), spondylolysis, NTD's and sacro-coccygeal fusion. Descriptive analysis was performed to determine the frequencies of defects in the selection. The descriptive analyses are illustrated in frequency distribution tables for each type of defect evaluated in the study. This study found that every specimen in the selection had TLTV and one or more additional random congenital defect in the vertebral column. Based on the finding, it can be claimed that an association exists between TLTV and other congenital defects of the vertebral column. TLTV were identified based on intermediary characteristics between the thoracic and lumbar regions present in the vertebra. This study concludes that when TLTV is present, it will be associated with one or more random defect in the vertebral column discussed in this study. The association between TLTV and other congenital defects provides an indirect association between all cases where various congenital defects are simultaneously present.

AFRIKAANSE OPSOMMING

Volgens Byrd & Comiskey (2016), wanneer ossifisering tydens ontwikkeling ontwrig word, lei dit tot abnormale skelet strukture. 'n Studie wat deur Masnicová & Beňuš (2003) voltooi was het tot die gevolgtrekking gekom dat meeste van die aangebore skeletgebreke in die vertebrale kolom geleë was. Die mees algemene skeletgebreke van die vertebrale kolom word deur ontwikkelingsagterstande van die vertebrale elemente veroorsaak (Masnicová & Beňuš 2003). In die literatuur meld gevalstuddies verskeie aangebore gebreke aan wat binne die vertebrale kolom van individue teenwoordig is. Daar is egter nie genoeg bewyse om te staaf of die waarnemings met mekaar assosieer kan word en of dit toevalig voorgekom het nie. Die doel van hierdie studie was om gebreke van 'n substel van gebreke in die vertebrale kolom vorm te identifiseer en om te evalueer of die gebreke met mekaar assosieer is. ‘n Seleksie van vertebrale kolomme (n = 35) is geneem uit 'n substel groep van die Kirsten skeletversameling by Stellenbosch Universiteit. Hierdie studie het voorspel dat daar ‘n assosiasie tussen verskeie aangebore gebreke in die vertebrale kolom is. Hierdie studie het bevind dat torakale en lumbale oorgangswerwels in al die skelete van die seleksie beskou kon word. Daar was, boonop, ten minste een ander addisionele aangebore afwyking in die vertebrale kolom van elke individu se skelet. Gebaseer op die bevinding, kom hierdie studie tot die gevolgtrekking dat 'n assosiasie tussen tarokale-lumbale oorgangs werwels en ander verskeie gebreke van die vertebrale kolom bestaan.

ACKNOWLEDGEMENTS

Gratitude is extended to the following individuals within the Anatomy Department of Stellenbosch University: my supervisor Mrs Linda Greyling and the head of department Professor Ben Page. Additional gratitude is extended to Mrs Amanda Alblas, a consultant and lecturer in the department. Lastly, I would like to thank Mr Reggie Williams for his support and technical guidance in the laboratory.

Appreciation and thanks are extended toward Mr Lourens Marthinus du Plessis, who aided in scribing during data collection.

I would like to sincerely thank Miss Beata Dongwi, a PhD student from Rhodes University, who provided consultation and guidance in this study.

Finally, sincere appreciation is extended to Dr Jana Jacobs. She provided statistical consultation and guidance to my project. The profound quality of her guidance was valuable beyond measure.

CONTENTS

FIGURES

TABLES

ABBREVIATIONS

BMP	Bone Morphogenic Proteins
C	Cervical vertebra
CNS	Central nervous system
CS	Cervical spondylolysis
EOS	Electro-optical system
f	Frequency
HOX	Homeobox gene
Hcy	Homocysteine
L	Lumbar vertebra
LSTV	Lumbosacral transitional vertebra
n	Number of specimens in the selection
N	Number of specimens in the population
NTD	Neural tube defects
ONTD	Open NTD's
S	Sacral vertebra
T	Thoracic vertebra
TLTV	Thoracolumbar transitional vertebra
µmole/L	Micro-moles per litre

INTRODUCTION

The vertebral column collectively refers to 33 vertebrae that are subdivided into five regions (Drake, Vogl & Mitchell 2009; Hansen 2010; Moore, Agur & Dalley 2010). Vertebrae vary in size and morphology from one region of the vertebral column to the other (Rawls & Fisher 2010). The vertebral column originates from the pre-somatic mesoderm under regulation of the notochord (Greene & Copp 2009). The development of the vertebral column takes place over the following phases: (1) gastrulation, (2) formation of the somatic mesoderm and notochord, (3) formation of dermomyotome and sclerotome from the somites, (4) re-segmentation of the somites to form the definitive vertebrae, (5) vertebral chondrification and (6) vertebral ossification (Dias 2007).

According to Byrd & Comiskey (2016), disrupted ossification during development results in abnormal skeletal development. A study conducted on congenital anomalies by Masnicová & Beňuš (2003), stipulated that most skeletal congenital defects were located in the vertebral column. The most common skeletal defects of the vertebral column are developmental delays of vertebral elements such as: neural tube defects (NTD's), spondylolysis and cranial-caudal border shifts (Masnicová & Beňuš 2003).

The clinical relevance varies among the defects. Infants that develop other NTD's have a high probability of developing severe lifelong disabilities (Wilson 2014). Spondylolysis is reported as a common cause of lower back pain and deteriorated quality of life in individuals (Attiah, Macyszyn & Cahill 2014; Metkar, Shepard, Cho & Sharan 2014; Wright, Balaji & Montgomery 2013). Lastly, cranial-caudal shifts of the vertebral column result in deviation from typical vertebral anatomy that can result in confusion and lead to significant clinical errors (Thawait, Chhabra & Carrino 2012).

The purpose of this study was to evaluate the frequencies of random congenital defects in the vertebral columns from a selection of skeletal remains. The frequencies were required to interpret whether associations are present between the random congenital defects that were observed.

Published literature has reported case studies of various simultaneous defects in the vertebral column mutually present in an individual. There is, however, a lack of evidence to substantiate whether the mutually inclusive observations resulted by chance, or whether an

association between the defects are present. In addition, controversy remains among researchers regarding the mechanisms that result in the fore-mentioned defects.

This study hypothesised that there is an association present between random congenital defects in the vertebral column that included: lumbosacral transitional vertebra (LSTV), thoracolumbar transitional vertebra (TLTV), spondylolysis, NTD's and sacro-coccygeal fusion.

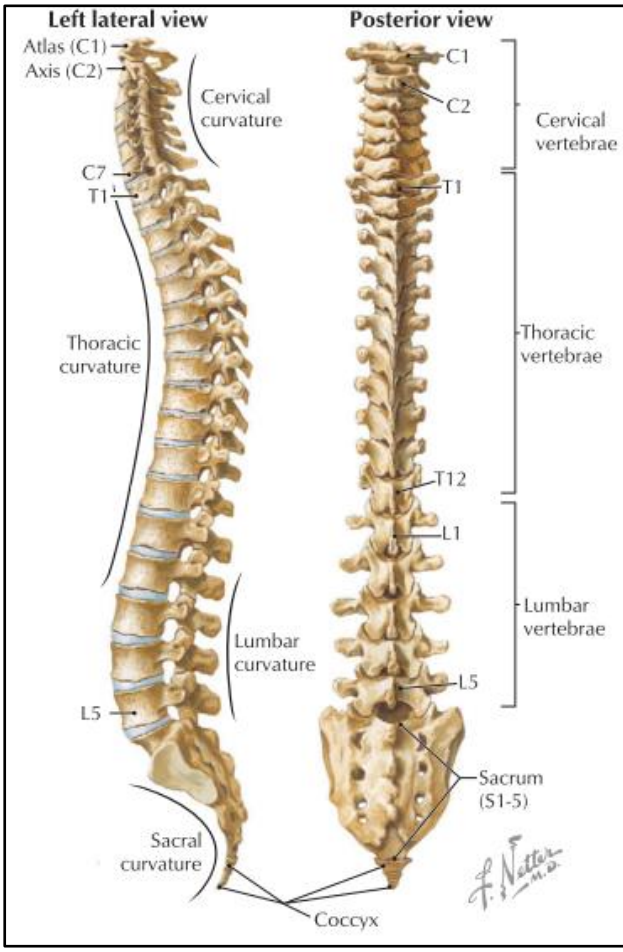
In this study, skeletal material from the Kirsten Skeletal Collection at Stellenbosch University Tygerberg Medical Campus was evaluated. A selection of specimens (n=35) with random congenital defects in the vertebral column was studied.

This study was a descriptive research study that determined the frequencies and associations present between observed defects in specimens from the selection.

LITERATURE REVIEW

1.1.GENERAL ORGANISATION OF THE VERTEBRAL COLUMN

The back is composed of skeletal, cartilaginous, ligamentous and muscular elements. Together the structures act as a flexible axis for the movement of the torso and transmit the weight of the body to the lower limbs. The vertebral column is the skeletal framework of the back and extends from the skull to the apex of the coccyx (Figure 1.1-1) (Botto, Moore, Khoury & Erickson 1999; Byrd & Comiskey 2016; Drake et al. 2009; Hansen 2010; Rawls & Fisher 2010).



Level	Corresponding structure
C2-3	Mandible
C3	Hyoid bone
C4-5	Thyroid cartilage
C6	Cricoid cartilage
C7	Vertebra prominens
T3	Spine of scapula
T8	Point of inferior vena cava pierces diaphragm
T10	Xiphisternal junction
T10	Point of esophagus entering stomach
T12	Point of aorta entering abdomen
L1	End of spinal cord
L3	Subcostal plane
L3-4	Umbilicus
L4	Bifurcation of aorta
L4	Iliac crests
S2	End of dural sac

Figure 1.1-1: Illustration of the Vertebral Column (Hansen 2010; Netter 2011)

The vertebral column collectively refers to 33 vertebrae that are subdivided into five regions (Figure 1.1-1). The five regions of the vertebral column are: the cervical, thoracic, lumbar, sacral and coccygeal regions (Botto et al. 1999; Byrd & Comiskey 2016; Drake et al. 2009; Hansen 2010; Moore et al. 2010; Rawls & Fisher 2010).

Vertebrae vary in size and morphology from one region of the vertebral column to the other. According to Moore et al. (2010), in each region the articular facets on the articular processes are orientated in a direction characteristic of that region that determines the type of movement permitted in that region (Botto et al. 1999; Drake et al. 2009; Hansen 2010; Rawls & Fisher 2010)

The cervical region forms the skeletal framework of the neck (Figure 1.1-1). There are seven vertebrae in the cervical region; five typical and two atypical. The atlas (C1) and axis (C2) are atypical cervical vertebrae (discussed in 1.2.2)(Drake et al. 2009; Moore et al. 2010; Oostra, Hennekam, De Rooij & Moorman 2005; Rawls & Fisher 2010).

Thoracic vertebrae form the midline of the posterior wall of the thoracic cavity (Figure 1.1-1). There are 12 thoracic vertebrae; corresponding to 12 pairs of ribs (discussed in 1.2.3)(Hansen 2010; Moore et al. 2010; Oostra et al. 2005; Rawls & Fisher 2010).

The five lumbar vertebrae form the skeletal support of the posterior abdominal wall (Figure 1.1-1). The five lumbar vertebrae are distinguished from vertebrae in other regions by their large size (discussed in 1.2.4)(Drake et al. 2009; Moore et al. 2010; Oostra et al. 2005; Rawls & Fisher 2010).

Unlike most other vertebrate mammals, humans do not have a tail. Instead, humans possess rudimentary coccygeal vertebrae at the caudal endpoint of the vertebral column. The coccygeal region lies adjacent to the sacral region in the vertebral column. Reviewed literature states that the number of coccygeal vertebrae can range between two to five vertebrae. The most frequent number of coccygeal vertebrae observed is four (discussed in 1.2.5)(Drake et al. 2009; Moore et al. 2010; Oostra et al. 2005; Rawls & Fisher 2010; Tague 2011b).

1.2.STRUCTURAL AND FUNCTIONAL ANATOMY OF VERTEBRAE

1.2.1. Anatomy of a typical human vertebrae

A typical vertebra is composed of a vertebral body and neural arch (Figure 1.2-1). The vertebral body is located anterior to the neural arch and articulates with adjacent intervertebral discs. The vertebral body is a weight bearing structure that increases in size relative to the mass that it has to support (Drake et al. 2009; Hansen 2010; Moore et al. 2010; Rawls & Fisher 2010). Vertebral bodies are composed of a core of trabecular bone and red marrow encased in cortical bone (Byrd & Comiskey 2016; Rawls & Fisher 2010)

The neural arch, also called the vertebral arch, is formed by right and left pedicles and laminae (Figure 1.2-1). The pedicle is a short strong process that fuses the neural arch to the vertebral body. Pedicles are fused to flat plate laminae posteriorly. The laminae unite in the midline of each vertebra (Byrd & Comiskey 2016; Drake et al. 2009; Hansen 2010; Kershenovich, Macias, Syed, Davenport, Moore & Lock 2015).

The spinous process is a posterior projection that originates from junction of the laminae in the midline (Figure 1.2-1)(Byrd & Comiskey 2016; Drake et al. 2009; Hansen 2010; Kershenovich et al. 2015).

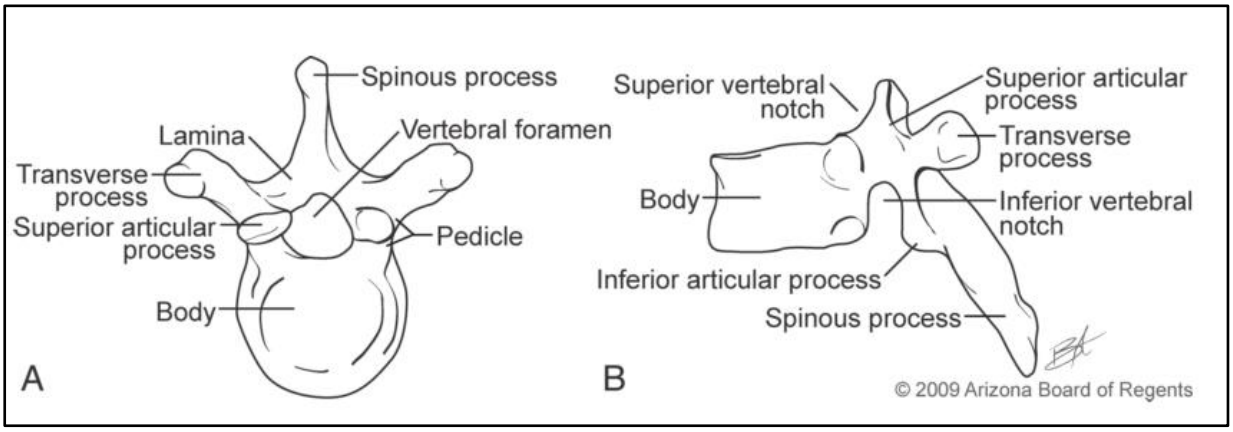


Figure 1.2-1: Features of a typical human vertebra (Rawls & Fisher 2010)

Each vertebra in the vertebral column is unique, but demonstrates characteristics that categorise them into one of the five regions (Botto et al. 1999; Drake et al. 2009; Hansen 2010; Rawls & Fisher 2010).

1.2.2. Anatomy of vertebrae in the cervical region

The first and second cervical vertebrae are atypical (Figure 1.2-2). The first cervical vertebra (C1) is referred to as the atlas. The atlas lacks a body; instead it has two lateral masses united by the posterior and anterior vertebral arch. In addition, C1 has no spinous process (Moore et al. 2010; Rawls & Fisher 2010).

The second cervical vertebra is called the axis (C2) (Figure 1.2-2). The axis does not have a typical vertebral body. The vertebral body of C2 is represented by an odontoid process called the dens. The dens articulates with the anterior neural arch of the axis at the median atlanto-axial joint (Moore et al. 2010; Rawls & Fisher 2010).

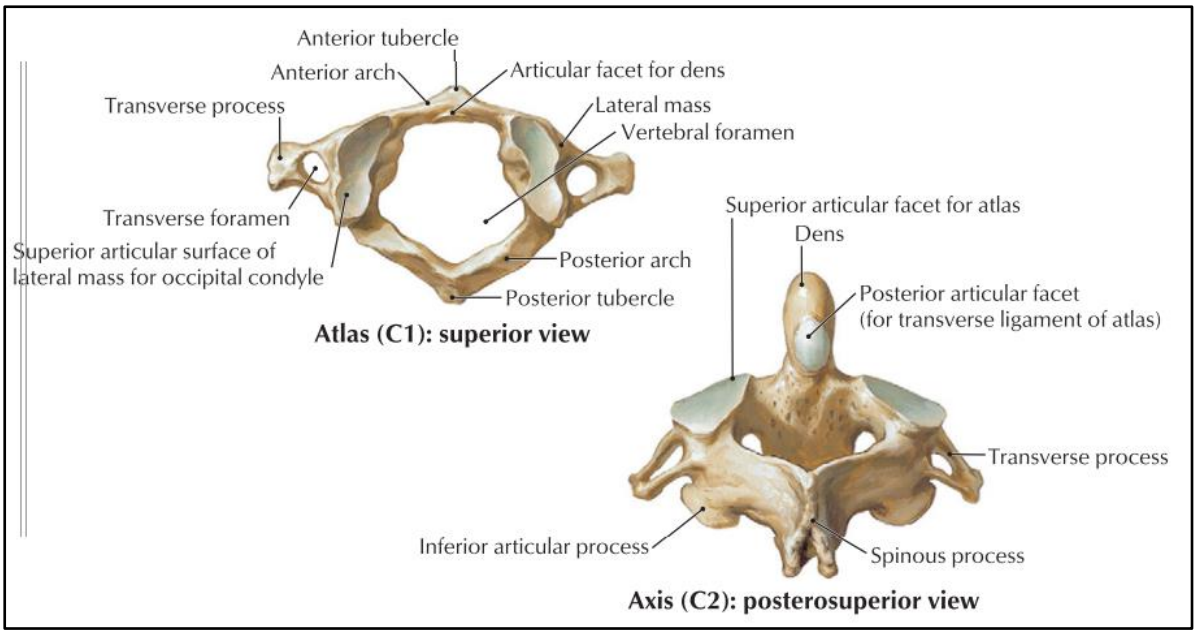


Figure 1.2-2: Structure of the atlas (C1) and axis (C2) (Hansen 2010; Netter 2011)

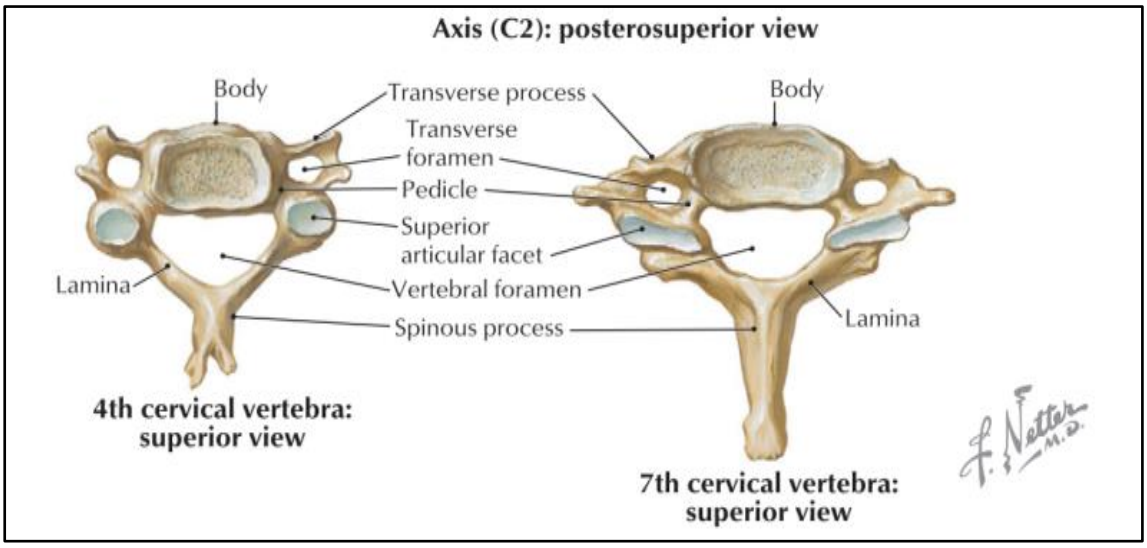


Figure 1.2-3: Illustration of typical cervical vertebrae (Hansen 2010; Netter 2011)

Typical cervical vertebrae have uncinate processes on the superior surface of the vertebral bodies (Byrd & Comiskey 2016; Drake et al. 2009; Kershenovich et al. 2015; Moore et al. 2010; Oostra et al. 2005; Rawls & Fisher 2010). The vertebral bodies of cervical vertebrae are relatively small, reflecting the minor weight bearing function (Rawls & Fisher 2010). The transverse processes of cervical vertebrae have foramina transversaria that permit vertebral arteries and veins to pass through. On occasion cervical vertebrae have been known to possess bifid spinous processes (Figure 1.2-3)(Drake et al. 2009; Rawls & Fisher 2010).

The direction of the superior and inferior articular facet orientation determines the function permitted in the region (Moore et al. 2010). In the cervical region, the superior articular facets face superior-posteriorly; and the inferior articular facets face antero-inferiorly (Figure 1.2-3). This promotes movements in the cervical joints that include flexion, extension, lateral flexion and rotation (Byrd & Comiskey 2016; Drake et al. 2009; Moore et al. 2010; Rawls & Fisher 2010; Oostra et al. 2005).

1.2.3. Anatomy of vertebrae in the thoracic region

A distinct feature of thoracic vertebrae is facets for costal articulation (Figure 1.2-4)(Rawls & Fisher 2010). A typical thoracic vertebra has two partial or hemi-facets on each side of the vertebral body for articulation with the head of its own rib and the head of the rib below (Drake et al. 2009; Moore et al. 2010; Rawls & Fisher 2010). The transverse process also has a facet for articulation with the tubercle of its own the same numbered rib. The joints between the ribs and vertebrae function to elevate and depress the ribs, thereby increasing the size of the thoracic cavity during respiration (Hansen 2010; Moore et al. 2010; Rawls & Fisher 2010). The eleventh (T11) and twelfth (T12) thoracic vertebrae are atypical as they do not have two hemi-facets on each side of the vertebral body. The vertebral body of T11 and T12 has one complete facet on each side (Moore et al. 2010).

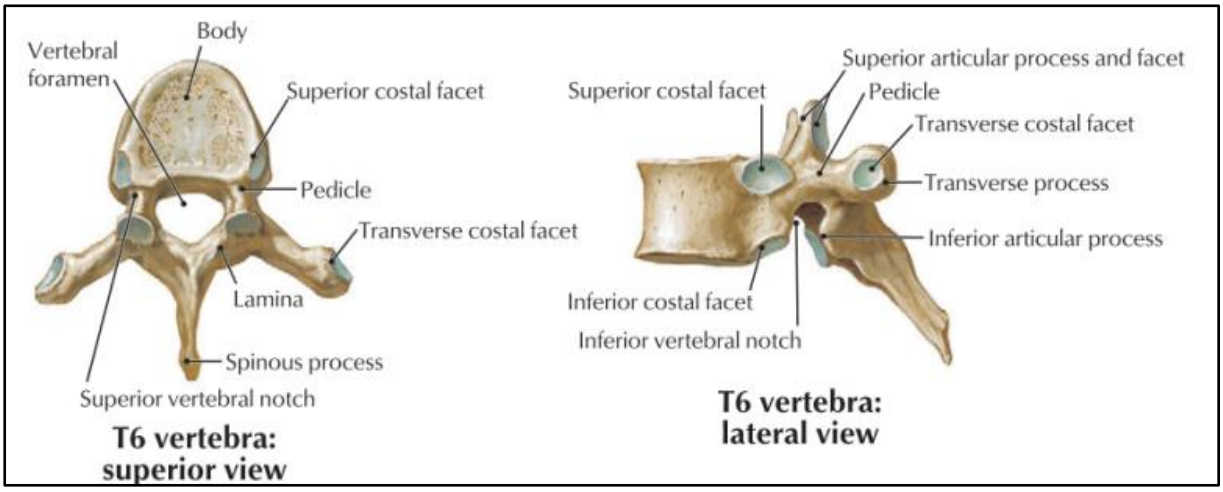


Figure 1.2-4: Illustration of a typical thoracic vertebra (Netter 2011; Hansen 2010)

The vertebral bodies of thoracic vertebrae are larger relative to the vertebral bodies in the cervical region. This represents the relatively higher weight bearing function of the thoracic region (Hansen 2010; Rawls & Fisher 2010).

In the thoracic region the superior articular facets are directed posteriorly, with a slight lateral angle. The inferior articular facets are directed anteriorly, and slightly medially (Drake et al. 2009; Moore et al. 2010; Rawls & Fisher 2010). The anterior and posterior orientations of the articular facets permit movements in the thoracic regions such as rotation and lateral flexion. The long inferiorly directed spinous processes of thoracic vertebrae restrict flexion and extension of the back in the thoracic region (Moore et al. 2010; Rawls & Fisher 2010).

1.2.4. Anatomy of vertebrae in the lumbar region

Vertebrae in the lumbar region are characterised by large vertebral bodies. The vertebral body of lumbar vertebra is typically cylindrical in shape. The vertebral foramen is triangular in shape and larger than thoracic vertebrae. The robust structure of the vertebral bodies in lumbar vertebrae provides strong weight bearing structures (section 1.1). There are no costal facets on lumbar vertebrae for articulation with ribs (Figure 1.2-5)(Drake et al. 2009; Moore et al. 2010; Rawls & Fisher 2010).

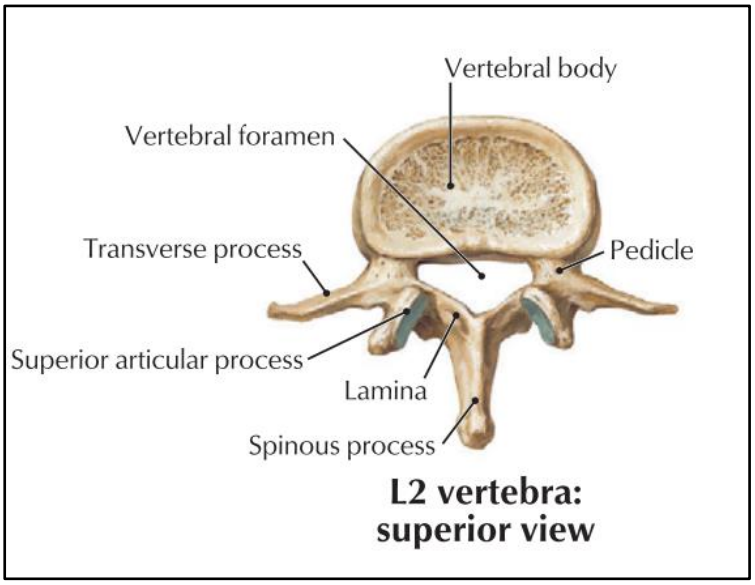


Figure 1.2-5: Illustration of typical lumbar vertebrae (Hansen 2010; Netter 2011)

The superior articular facets of lumbar vertebrae are directed medially, at a mild posterior angle. The inferior articular facets are directed laterally, at a mild anterior angle (Figure 1.2-5). The medially and laterally orientated articular facets of lumbar vertebrae permit a large degree of flexion and extension of the back in the lumbar region (Moore et al. 2010; Rawls & Fisher 2010).

Lumbar vertebrae have unique mammillary processes on the lateral surface of the superior articular processes. The transverse processes are generally thin and long, with the exception of the L5. The fifth lumbar vertebra has large transverse processes with accessory processes for attachment of iliolumbar ligaments that connect the transverse processes to the pelvic bones. It is suggested that the long slender transverse processes of lumbar vertebra are homologs of the thoracic ribs (Drake et al. 2009; Hansen 2010; Moore et al. 2010).

1.2.5. Anatomy of the sacrum and coccyx

The segments that compose sacral vertebrae are fused to form the sacrum (Figure 1.2-6). The sacrum transmits the weight of the trunk of the body to the legs via the pelvic girdle. The sacrum articulates to the pelvic bone at the sacroiliac joints. Superiorly, the sacrum articulates with the last lumbar vertebra (L5) at the base of the sacrum, which is formed by the superior surface of S1.The sacrum is characterised by four pairs of sacral foramina on the pelvic and dorsal sides. Three vertical crests are visible on the dorsal surface of the sacrum (Drake et al. 2009; Moore et al. 2010; Rawls & Fisher 2010). The spinous processes of the sacral vertebrae are fused to form the medial sacral crest. The fused articular processes form the intermediate sacral crest and the fused transverse processes form the lateral sacral crest. The distal sacral vertebrae lack laminae, forming the sacral hiatus. The articular process of the last sacral vertebra extends downward forming sacral cornua (Oostra et al. 2005).

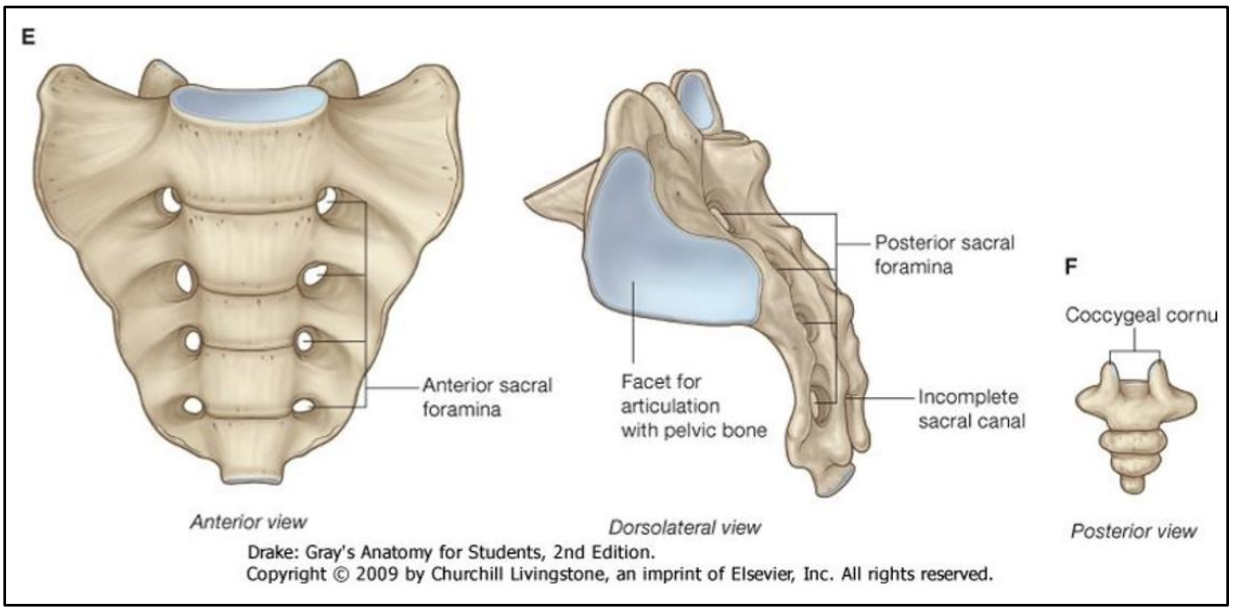


Figure 1.2-6: The anterior & dorsolateral view of the sacrum; and posterior view of the coccyx (Drake et al. 2009)

The coccygeal region forms the apex of the sacrum. It consists of four coccygeal vertebrae that are fused (Figure 1.2-6)(Drake et al. 2009; Moore et al. 2010; Oostra et al. 2005; Rawls & Fisher 2010).

Despite the minute relative size of the vertebrae in the coccygeal region, the vertebrae retain function in humans. The coccygeal bones provide area for muscle attachment in the pelvic cavity for muscles such as the gluteus maximus, levator ani, coccygeus and sphincter ani externus muscles. In addition, ligaments attach to the vertebrae of the coccyx such as the sacrospinous and sacrotuberous ligaments (Drake et al. 2009; Hansen 2010; Moore et al. 2010; Rawls & Fisher 2010; Tague 2011b).

1.3.DEVELOPMENT OF THE SPINE

1.3.1. Early human embryonic development

Human development starts after fertilisation when two haploid gametes fuse to form a diploid zygote. The zygote undergoes proliferation to form a morula and, ultimately, a blastocyst (Figure 1.3-1)(Dias 2007; Oostra et al. 2005). The blastocyst is a bilaminar embryo suspended between the amniotic and yolk sacs (Figure 1.3-1: B)(Dias 2007).

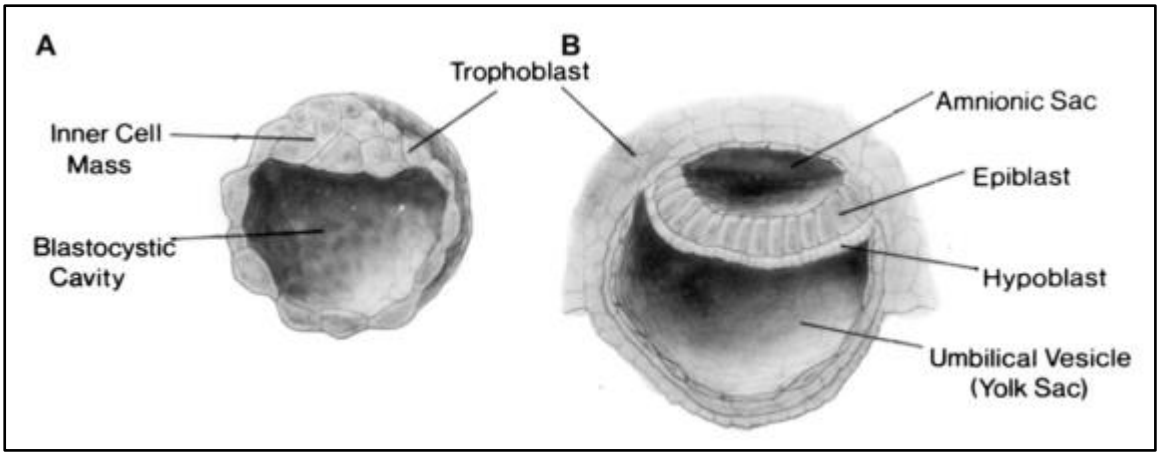


Figure 1.3-1: Illustrations- A: blastocyst and B: bilaminar embryo (Dias 2007)

The inner cell mass is transformed into a bilaminar structure that consist of two cell layers. The two cells layers are called the epiblast–located on the dorsal surface- and hypoblast–located on the ventral surface (Figure 1.3-1) (Dias 2007; Oostra et al. 2005).

During the second to third week of development, gastrulation takes place (Figure 1.3-2). Gastrulation is defined as the process that transitions the bilaminar embryo into a trilaminar embryo. There are three cell layers in the trilaminar embryo that are present after gastrulation,

called the: ectoderm, mesoderm, and endoderm (Oostra et al. 2005). Gastrulation takes place when a midline primitive streak develops at the caudal end of the embryo. Epiblast cells of the bilaminar layer migrate toward the primitive streak and move through the primitive groove (Figure 1.3-1). During gastrulation, coordinated cell movement occurs at the primitive streak to form the trilaminar embryo (Dias 2007).

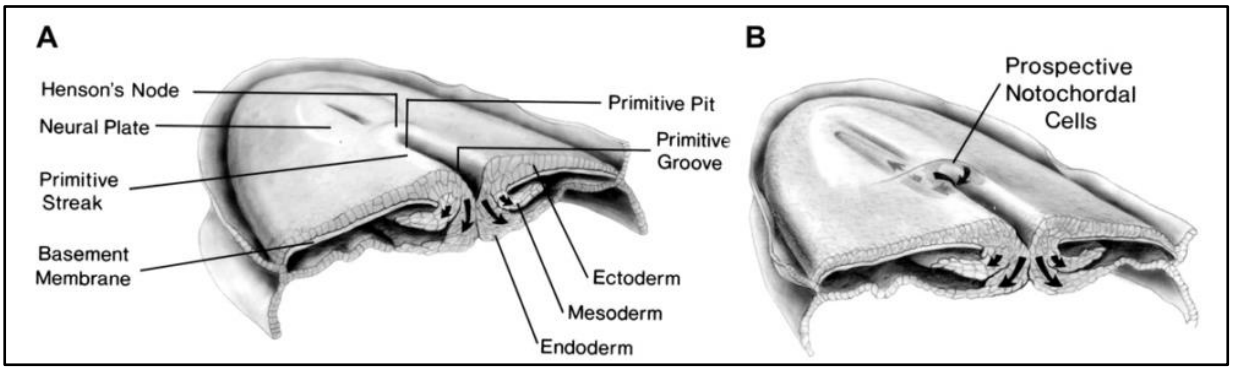


Figure 1.3-2: Illustration of gastrulation (Dias 2007)

The next important structure that is formed is called the notochord. At the cranial end of the primitive streak is Hensen's node (Figure 1.3-2: A). Within Hensen's node is an extension of the primitive groove which is called the primitive pit. Cells within Hensen's node move through the primitive pit to form the midline notochord. The notochord is located in the centre of the mesoderm. The notochord is a signalling structure that will signal the, mesoderm and endoderm to form all organs and related systems (Figure 1.3-2)(Byrd & Comiskey 2016; Dias 2007).

The ectoderm of the trilaminar embryo will form the skin and central nervous system (CNS) The mesoderm will form all the connective tissue and muscular structures. The endoderm layer will form the internal lining of the gastrointestinal, respiratory and urogenital tracts. The notochord ultimately develops into the nucleus pulposus of intervertebral discs (Dias 2007; Greene & Copp 2009; Khairnar & Rajale 2013; McMahon, Takada, Zimmerman, Fan, Harland & McMahon 1998; Oostra et al. 2005).

1.3.2. Development of the CNS

The CNS starts to develop in the third week of embryological growth; appearing as the neural plate. The neural plate originates from the ectoderm of the trilaminar embryo. The neural plate is located rostral relative to the primitive node within the mid-dorsal region. The first signal that the notochord sends initiates a morphological process called neurulation.

Neurulation is defined as a series of coordinated morphological events that convert the flat neural plate into the primordium of the CNS called the neural tube (Figure 1.3-3)(Greene & Copp 2009; Thawait et al. 2012).

Neurulation is subdivided into two phases: primary and secondary neurulation. Primary neurulation defines the development of the neural tube that will ultimately be the precursor of the brain and the spinal cord. Primary neurulation starts approximately 3-4 weeks after fertilisation has occurred, forming the brain and neural tube. The lateral edges of the neural plate elevate to form the neural folds. As the folds progressively elevate, the neural folds migrate medially and fuse to form the neural tube (Figure 1.3-3). Upon initiation, fusion of the neural tube begins in the cervical region; migrating to the cephalic and caudal ends. Initiation of fusion induces the formation of the cranial and caudal neuropores (Botto et al. 1999; Greene & Copp 2009; Puvirajesinghe & Borg 2015).

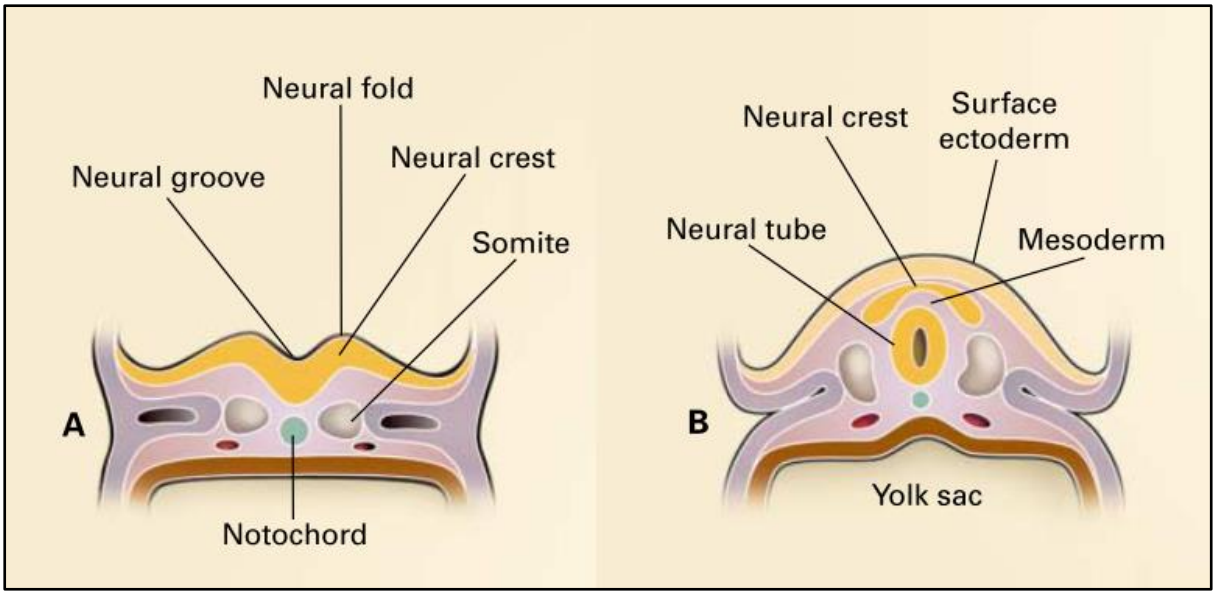


Figure 1.3-3: Neural tube formation (Botto et al. 1999)

Alternatively, secondary neurulation involves the condensation of a population of mesenchyme cells to form an epithelial rod. Neuroepithelial cells give rise to primitive nerve cells called neuroblasts. Neuroblasts develop into a primitive nerve cell layer called the mantle layer around the neuroepithelial layer. Ultimately, the mantle layer matures into the grey matter of the spinal cord. Conversely, the white matter of the spinal cord is located in the outermost layer, primordially called the marginal layer. White matter contains nerve fibres emerging from neuroblasts in the mantle layer; appearing white as a result of myelination of the nerve fibres (Puvirajesinghe & Borg 2015; Greene & Copp 2009).

1.3.3. Development of the vertebral column

Equally important as the development of the CNS is the embryological development of the neurocranium and vertebral column; the main function of which is to protect vital nervous tissue (Masnicová & Beňuš 2003).

The development of the vertebral column takes place over the following phases: (1) gastrulation, (2) formation of the somatic mesoderm and notochord, (3) formation of dermomyotome and sclerotome from the somites, (4) re-segmentation of the somites to form the definitive vertebrae, (5) vertebral chondrification and (6) vertebral ossification (Dias 2007).

Gastrulation takes place during early embryonic development. The mesoderm of the trilaminar embryo differentiates into a subdivided mesoderm. The structures in the mesoderm are the: pre-somatic or paraxial mesoderm (somites); the intermediate mesoderm (gonads and kidneys) and the lateral plate mesoderm (Dias 2007; Drake et al. 2009; Greene & Copp 2009; McMahon et al. 1998; Oostra et al. 2005; Rawls & Fisher 2010).

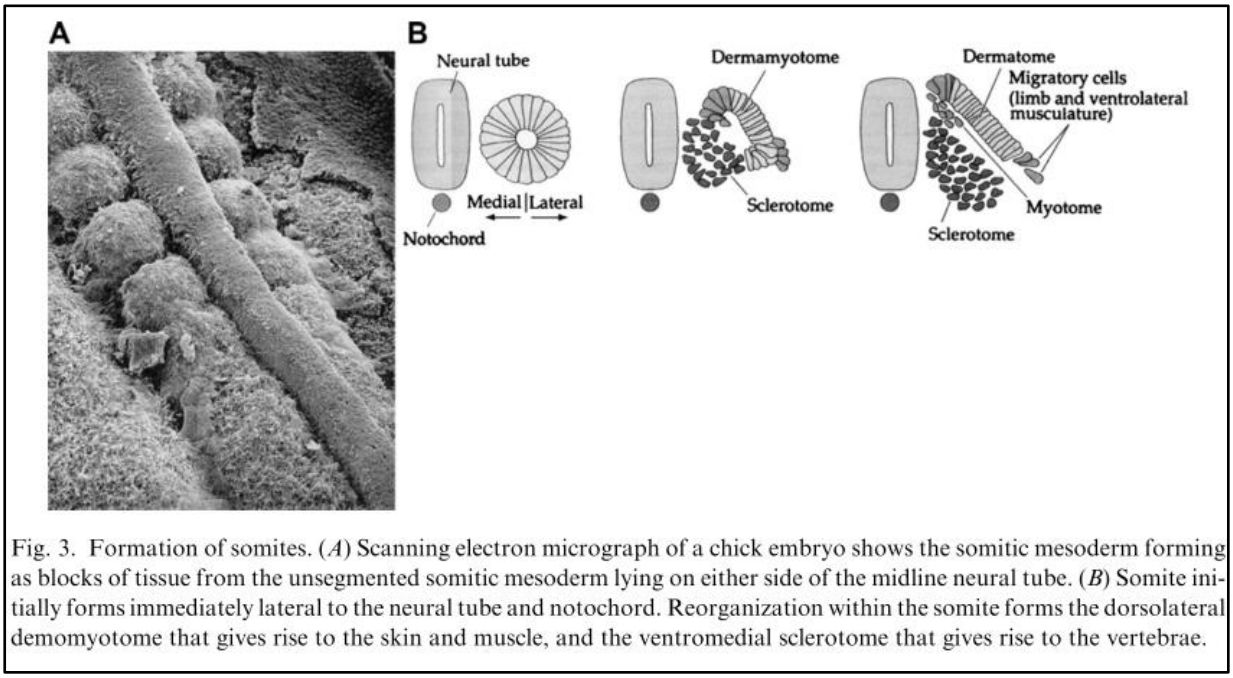


Fig. 3. Formation of somites. (*A*) Scanning electron micrograph of a chick embryo shows the somitic mesoderm forming as blocks of tissue from the unsegmented somitic mesoderm lying on either side of the midline neural tube. (*B*) Somite initially forms immediately lateral to the neural tube and notochord. Reorganization within the somite forms the dorsolateral demomyotome that gives rise to the skin and muscle, and the ventromedial sclerotome that gives rise to the vertebrae.

Figure 1.3-4: Formation of Somites (Dias 2007)

The vertebral column originates from the pre-somatic (paraxial) mesoderm under regulation of the notochord. Other structures that originate from the paraxial mesoderm are the muscles and associated tendons in the back. There are two layers of paraxial mesoderm that are formed longitudinally on either side of the neural tube during gastrulation (Figure 1.3-4).

Twenty days after fertilisation has taken place, the paraxial mesoderm undergoes segmentation. Segmentation takes place in a rostral to a caudal direction. During segmentation, the paraxial mesoderm is divided into 42-44 pairs of somites. Somites are formed when mesenchymal cells of the paraxial mesoderm continuously separate from the longitudinal paraxial mesoderm (Figure 1.3-5)(Greene & Copp 2009; Rawls & Fisher 2010; Thawait et al. 2012).

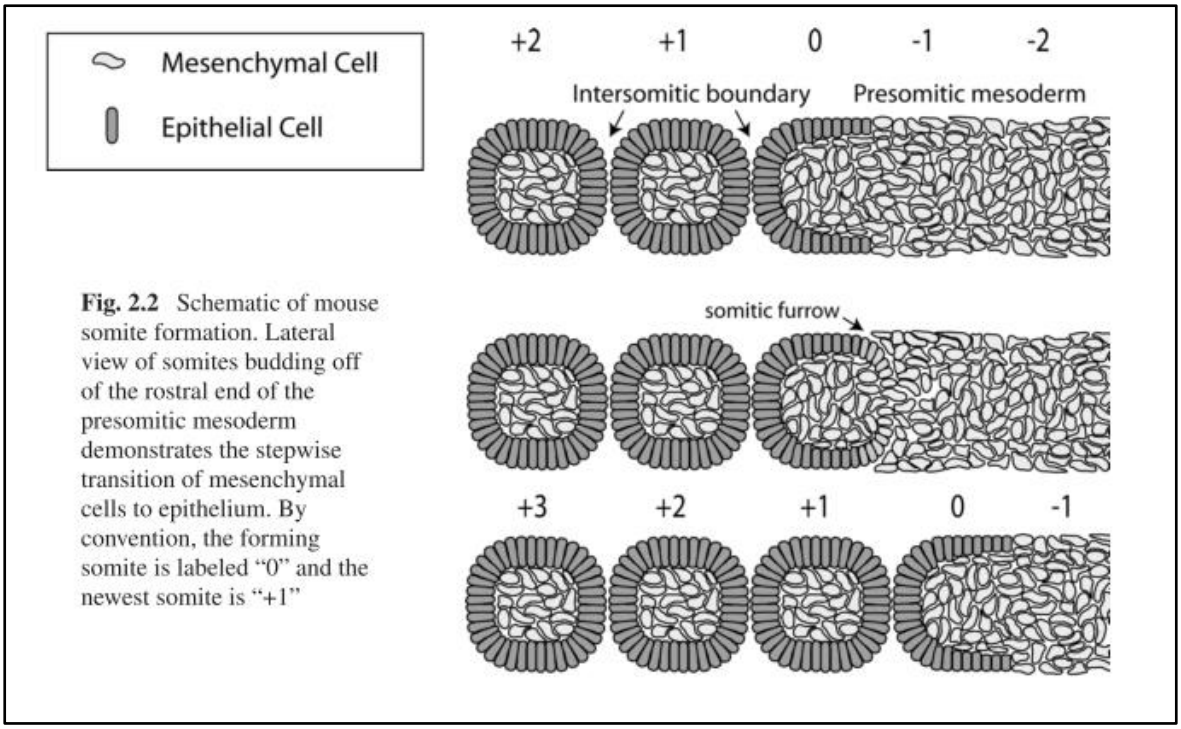


Fig. 2.2 Schematic of mouse somite formation. Lateral view of somites budding off of the rostral end of the presomitic mesoderm demonstrates the stepwise transition of mesenchymal cells to epithelium. By convention, the forming somite is labeled "0" and the newest somite is "+1"

Figure 1.3-5: Illustration of somitogenesis and epithelialisation (Rawls & Fisher 2010)

Somite formation is regulated by an intrinsic process that controls the timing of somitogenesis. For a somite to develop, an intersomitic boundary must be established. Boundary formation occurs when the somitic cells of the somite pull apart from the paraxial mesoderm. A somitic furrow -or fissure -is formed where the somite separates from the presomitic mesoderm (Figure 1.3-5)(Dias 2007; Rawls & Fisher 2010; Thawait et al. 2012).

Cells from the newly formed somites proliferate and increase the amount of extracellular matrix proteins in the cytoplasm. The increased matrix proteins increase the density of the somitic cells. The somite transforms into a somitocoel. The somitocoel is a rounded structure with a mesenchyme core enveloped by epithelial cells (Figure 1.3-5). Epithelialisation is complete with the formation of the next boundary and is called epithelial to mesenchyme

transition. Somite epithelialisation is associated with an increase in the expression of members of cell adhesion molecules such as the cadherin superfamily (Rawls & Fisher 2010).

The 42-44 pairs of somites that form can be divided into: four occipital, eight cervical, 12 thoracic, five lumbar, five sacral, and eight to ten coccygeal somites. The first occipital and last five coccygeal somites disappear during embryonic development (Rawls & Fisher 2010). The first four and a half cranial somites form the occipital bone. The second half of the fifth somite develops into the posterior arch of the atlas. The remainder of the cervical vertebrae are formed by somites six to 12. The thoracic vertebrae differentiate from somites 12-23 and 23-24. The lumbar vertebrae develop from somites 24-25; 26-27 and 28-29. The sacrum develops from somites 29-30 and 30-34. The coccyx differentiates from somites 34-35; 36-38 and 39-40 (Bauer et al. 2002; Carrino, Campbell, Lin, Morrison, Schweitzer, Flanders, Eng & Vaccaro 2011; Drake et al. 2009; Khairnar & Rajale 2013; Oostra et al. 2005; Rawls & Fisher 2010).

Somites define the paraxial mesoderm into various primordial vertebral regions. Each somite will differentiate into four compartments that are lineage specific. This occurs in the fourth week of gestation. The four lineage specific compartments are called the: sclerotome, syndetome, myotomes and dermomyotome. The sclerotome will form the vertebrae and the corresponding ribs. The myotome will form the skeletal muscle and the syndetome will form the associated tendons of the muscles. The dermomyotome will develop into the dermis and skeletal muscle progenitor cells (Dias 2007; Masnicová & Beňuš 2003; Rawls & Fisher 2010).

At the time when a somite is formed, rostral and caudal polarity of the somite is established (Figure 1.3-6). This an important step to facilitate segmental patterning of the peripheral nerves and re-segmentation of the sclerotome during vertebrae formation (Copp, Stanier & Greene 2013; Greene & Copp 2009; Rawls & Fisher 2010).

The development of vertebrae requires the migration of sclerotome cells along the rostral/caudal axis and toward the midline. Cells from the medial sclerotome migrate toward the notochord where they will form part of the intervertebral disc and vertebral body. Both halves of the sclerotome of adjacent somites contribute equally to the vertebral body. Subsequently, the lateral sclerotome cells migrate dorsally to form the vertebral pedicles and the laminae of the neural arches. The neural arches are derived from the caudal part of the somite and the spinous from the rostral part. The rostral and caudal half of the sclerotome can

be distinguished by their density (Figure 1.3-6) (Copp et al. 2013; Greene & Copp 2009; Rawls & Fisher 2010).

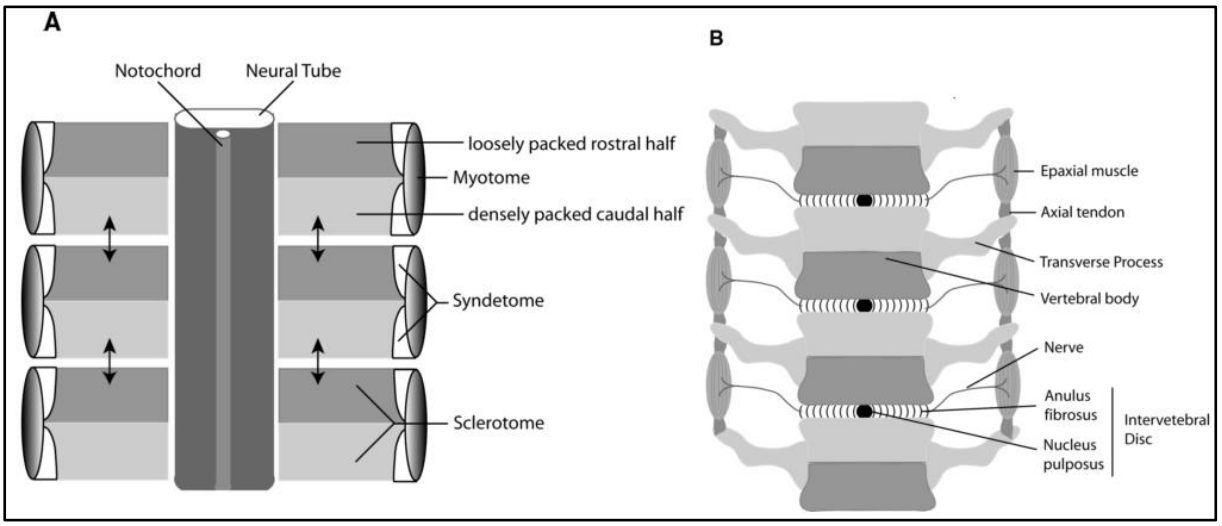


Figure 1.3-6:Illustration of the syndetome, sclerotome and myotome compartments (Rawls & Fisher 2010)

The axial skeleton originates from the sclerotome compartment of the somites. Vertebrae are formed from the sclerotome cells through the process of endochondral ossification (Dias 2007; Rawls & Fisher 2010; Thawait et al. 2012). Chondrification takes place during the sixth week of embryonic development. Chondrification starts at the cervical and thoracic region and extends cranially and caudally through the vertebral column. There are three pairs of chondrification centres that appear for each vertebra. The first pair forms within the vertebral centrum. The second pair forms dorso-laterally within the posterior vertebral arches and spinous process. The third pair forms within the transverse and costal arch. Chondrification of the sacrum and coccyx is similar to chondrification of vertebrae in other regions (Byrd & Comiskey 2016; Dias 2007).

The final step in vertebral development is vertebral ossification. Ossification of vertebrae starts during the eighth week of embryonic development and continues during infancy. There is much controversy regarding the number of ossification centres that form. Some authors state that three ossification centres develop in each vertebra. Other authors suggest that as many as six ossification centres may be present. Ossification starts at the thoracolumbar junction (T10-L1) (Dias 2007). Ossification continues to T2 and L4 and proceeds in a bidirectional fashion through the vertebral column. The vertebral bodies in infants are oval in form through the entire vertebral column. In addition, the height of the intervertebral discs

and vertebral bodies are similar. At the ages of two to three years-of-age, the vertebral bodies assume a rectangular shape (Byrd & Comiskey 2016). There is a coronal cleft in the vertebral body during the first six to 12 months of infancy. The cleft is completely fused by the ages of two to three years as the vertebral body is ossified. The ossified vertebral junction body is separated from the vertebral arch. There are neurocentral synchondroses at the junction of the neural arch and vertebral body. The primary ossification centres of the neural arch are present. The laminae are not yet fused (Figure 1.3-7) (Byrd & Comiskey 2016; Dias 2007; Moore et al. 2010).

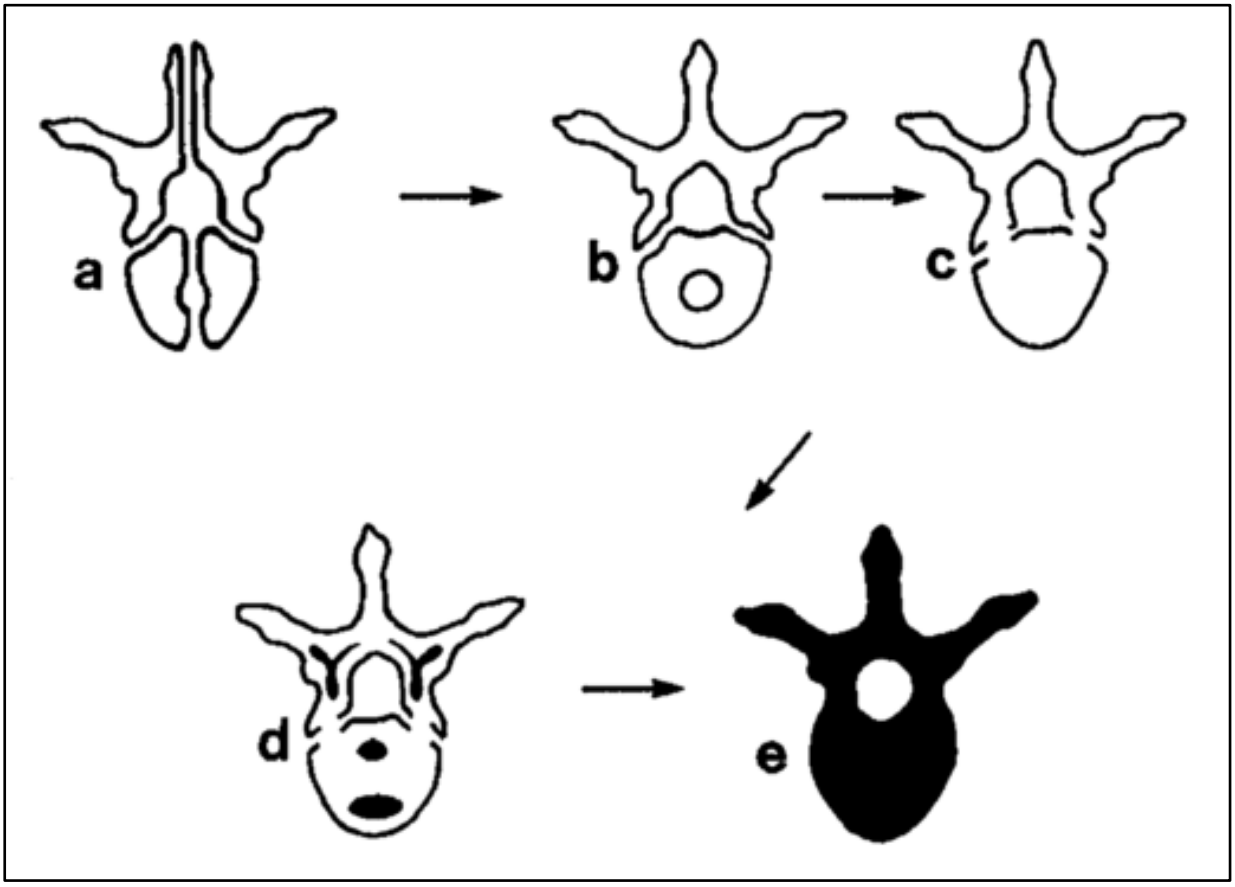


Figure 1.3-7: Normal development of the vertebrae (Byrd & Comiskey 2016)

The last process that changes the shape and size of vertebrae is the formation of secondary ossification centres. The primary and secondary ossification centres fuse at 15-16 years postnatal development. The secondary ossification of vertebrae is complete between the ages of 18 and 25 (Dias 2007; Masnicová & Beňuš 2003; Savage 2005). The secondary ossification centres are located in the spinous processes, transverse processes and the ring apophysis (Byrd & Comiskey 2016; Thawait et al. 2012).

The difference between the sacrum and other vertebral regions is that the first three sacral vertebrae contain an additional pair of ossification centres (Dias 2007). Ossification of the first sacral vertebral takes place between the ages of one and four. Fusion of the sacral vertebrae starts in puberty. The coccygeal vertebrae ossify between the ages of five and 20 years-of-age. The coccyx often remains segmented, although fusion may occur (Dias 2007).

The vertebral column continues to mature postnatally. Changes occur predominantly in vertebrae during infancy to early adolescence. Maturation of the vertebral column includes ossification of vertebrae, changes in the shape of the vertebrae, formation of spinal curvatures, changes of the intervertebral discs and changes in the bone marrow (Byrd & Comiskey 2016).

Adults have vertebral columns with four curvatures called the: cervical, thoracic, lumbar and sacral curvatures (Figure 1.1-1). The curvatures in the vertebral column provide flexible support for the body and absorb shock associated with movement. The thoracic and sacral curvatures are concave anteriorly and are referred to as primary curvatures. Primary curvatures are formed during foetal development and are retained throughout the life of the individual. The cervical and lumbar curvatures are secondary curvatures. The secondary curvatures are concave posteriorly. Secondary curvatures begin to form during foetal development, but do not complete formation until infancy. The cervical curvature becomes prominent when an infant begins to support their heads. The lumbar curvature becomes prominent when an infant begins to support the trunk in an upright position (Byrd & Comiskey 2016; Moore et al. 2010).

Additional regulation is needed to apply the distinctive regional characteristics between vertebral regions. Members of the homeobox (HOX) transcription factor family have been strongly implicated in regional identity of vertebrae along the rostral/caudal axis. The regional identity conferred by HOX genes during vertebral patterning is modified by the polycomb family of homeodomain containing transcription factors (Forseen, Gilbert, Patel, Ramirez & Borden 2015; Rawls & Fisher 2010; Thawait et al. 2012).

1.4.ABNORMALITIES OF THE VERTEBRAL COLUMN

A study conducted on congenital anomalies by Masnicová & Beňuš (2003) concluded that most skeletal congenital defects were located in the vertebral column. The most common skeletal defects of the vertebral column are developmental delays of vertebral elements such as: NTD’s, spondylolysis and cranial-caudal border shifts (Masnicová & Beňuš 2003).

It is suggested that pleiotropic effects of HOX mutations lead to abnormalities in the vertebral column (Asher, Lin, Kardjilov & Hautier 2011; Thawait et al. 2012).

1.4.1. NTD's

The theory that NTD's results from defective closure of the posterior neural tube, between 21 and 28 days of prenatal development, is repeated in reviewed literature (Ahmad & Mahapatra 2009; Botto et al. 1999; Puvirajesinghe & Borg 2015; Wilson 2015).Various postulations have been made regarding the normal closure of the neural tube during neurulation. Currently, the two most accepted theories include: (1) the single-site closure theory and (2) the multiple-site closure theory (Figure 1.4-1). Both theories contain significant insight and have been thoroughly debated, although indisputable evidence is insufficient to substantiate either theory as fact (Ahmad & Mahapatra 2009).

The single-site closure theory states that closure of the neural tube originates from a single location and moves bi-directionally to complete closure (Figure 1.4-1). Insufficient closure at the extreme locations of the posterior neuropores results in spina bifida. According to this theory, NTD's are limited to only the cervical and lumbo-sacral regions. There are, however, a variety of recorded NTD's in reviewed literature that contradicts this postulation. It may occur that NTD's are located at any of the regional junctions (Ahmad & Mahapatra 2009; Martínez-Frías, Urioste, Bermejo, Sanchís & Rodríguez-Pinilla 1996; Tekkök 2005).

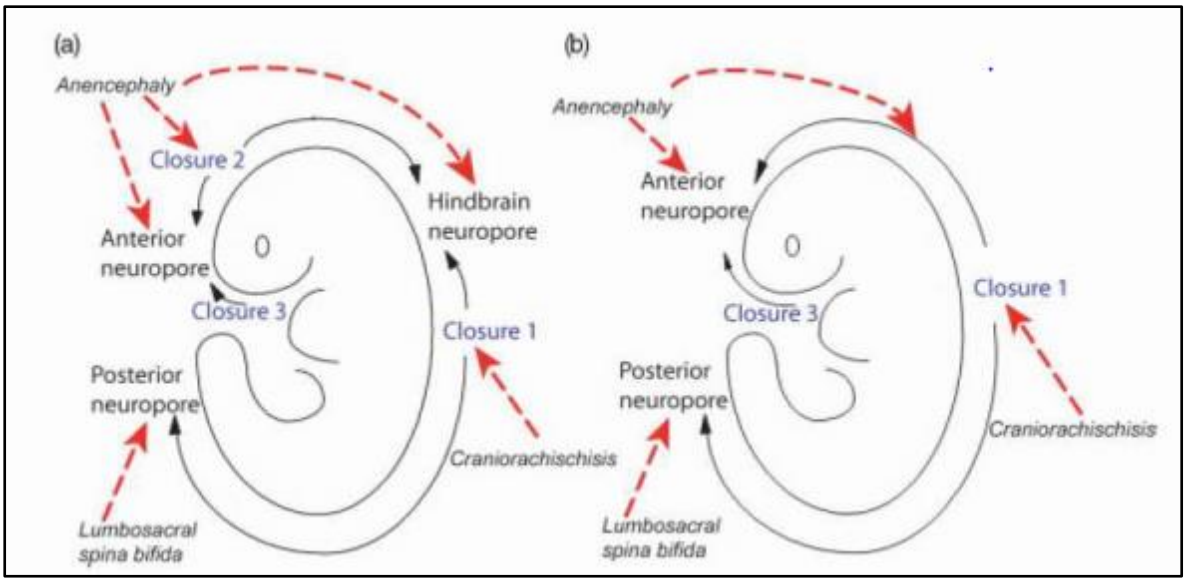


Figure 1.4-1: Closure of the posterior neuropores in embryos (Greene & Copp 2009)

Contrarily, the multiple-site closure theory states that there are many sites that initiate neural tube closure (Figure 1.4-1). Several theories have been postulated in favour of this, including theories from Nakatsu et al. (2000), O'Rahally and Muller (2002) and Van Allen et al. (1993). According to Van Allen (1993), there are five closure sites in the neural tube. NTD's can result from defective closure of any of the five sites. According to a study conducted by Ahmad and Mahapatra (2009), the multiple-site closure theory of Van Allen was the only theory that was fully able to explain the case reports of multiple NTD's found in the study (Ahmad & Mahapatra 2009; Martínez-Frías et al. 1996; Tekkök 2005).

Disruption of primary neurulation results in "open" NTD's, and disruption of secondary neurulation results in "closed" NTD's. Supporting the multiple-site closure theory, it postulates that three anatomically distinct closure sites are identified in mammals (Puvirajesinghe & Borg 2015; Greene & Copp 2009).

Disruptive neural tube closures are classified according to two sites namely: the anterior and posterior neuropore. Failure of anterior neuropore fusion is most prevalent in the superior vertebral regions resulting in anencephaly and encephalocele. Conversely, defective closure of the posterior neuropore may cause in defects such as spina bifida and meningomyocele; predominantly located in the inferior vertebral regions (Bauer et al. 2002; Puvirajesinghe & Borg 2015; Wilson 2014; Wilson 2015)

Foetuses that develop anencephaly do not survive; they are spontaneously aborted or stillborn (Botto et al. 1999; Copp et al. 2013; Puvirajesinghe & Borg 2015). Infants who develop other NTD's have a high probability of developing severe lifelong disabilities (Wilson 2014). Individuals may be neurologically deficit, develop endocrine abnormalities, have deformations of the spinal column, suffer from learning disabilities and sexual, bladder or bowel dysfunction. In developed countries, such as the United States, 90% of infants born with a NTD live beyond one year; although they continue to experience increased deterioration and medical complications. In underdeveloped countries medical care and prenatal screening is not freely available. This often prevents effective treatment (Botto et al. 1999; Puvirajesinghe & Borg 2015; Taylor, Farkouh, Graham, Colligs, Lindemann, Lynen & Candrilli 2011).

Spina bifida refers to an assembly of congenital defects resulting from the impaired closure of the posterior neuropore. There is subsequent disruption of vertebral arches and the axial mesoderm. Locations where skeletogenesis are disrupted prohibit neuroepthileium from

protecting underlying sclerotome, leaving the midline exposed. The risk of early death among infants with spina bifida depends on the severity of the lesions, although many infants with spina bifida are known to survive. Spina bifida is classified into three main types namely: spina bifida occulta, meningocele and myelo-meningocele. Meningocele and myelo-meningocele are often collectively referred to as spina bifida cystica. Spina bifida occulta is a skeletal defect of the first or second sacral vertebra often covered by a layer of skin (Copp et al. 2013; Masnicová & Beňuš 2003; Botto et al. 1999; Moore et al. 2010; Tekkök 2005). A meningocele is a saccular herniation of meninges and cerebrospinal fluid (Figure 1.4-2). Myelo-meningocele is the most common type of spina bifida and is characterised by herniation of the spinal cord, nerves, or both through a bony defect of the spine (Moore et al. 2010).

It is uniformly stated among reviewed literature that NTD's are etiologically heterogeneous and that both genetic and environmental factors contribute to the cause (Bauer et al. 2002; Greene, Stanier & Copp 2009; Myrianthopoulos & Melnick 1987; Puvirajesinghe & Borg 2015).

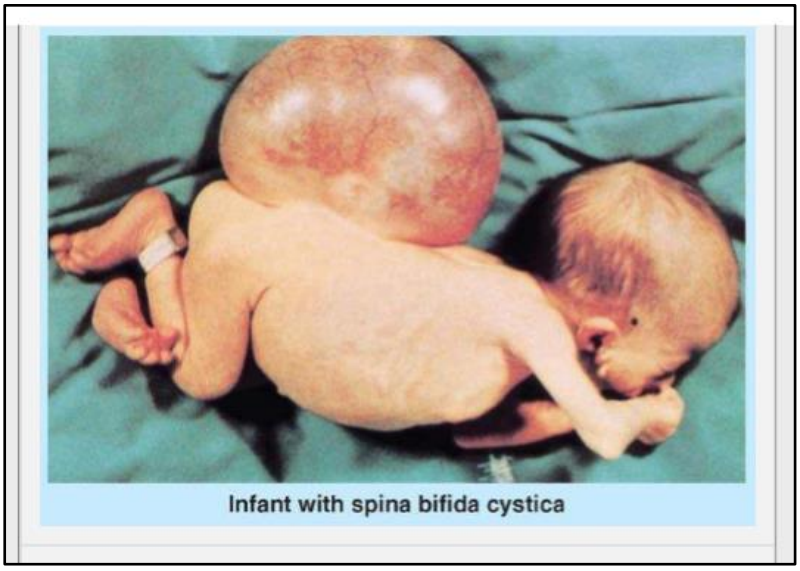


Figure 1.4-2: Illustration of infant with spina bifida cystica (Moore et al. 2010)

External factors associated with the development of NTD's include: a lack of dietary supplementation, teratogenic medication exposure, drugs, smoking, glucose metabolism, gastrointestinal absorption and alcohol (Wilson 2015). A study conducted by Shaw et al. (2009), evaluated the teratogenic pre-conception effects of smoking on NTD's in infants. The study found that smoking increased the risk of cleft lips, but seemed to reduce the risk of

NTD's. It was concluded that mixed findings are observed based on conclusions in previous literature (Shaw et al. 2009).

Maternal factors during gestation that have been reported to be significantly associated with NTD offspring include: diabetes mellitus, organic heart disease and lung disease. In addition, the maternal use of diuretics, antihistamines and sulphonamide are associated with NTD offspring (Myrianthopoulos & Melnick 1987; Botto et al. 1999).

Maternal supplementation of vitamin B12 and folic acid was shown to significantly reduce congenital anomalies, such as NTD's in infants (Botto et al. 1999; Mobasheri, Keshtkar & Golalipour 2010; Puvirajesinghe & Borg 2015; Taylor et al. 2011). Folic acid is the synthetic form of folate (Shaefer 2015). Women with low micro-nutrient and vitamin serum concentrations have a high probability of giving birth to babies with NTD's (Botto et al. 1999; Mobasheri et al. 2010; Puvirajesinghe & Borg 2015; Wilson 2015). Other benefits of folic acid and vitamin B12 supplementation include prevention of congenital anomalies such as: limb defects, heart defects, urinary tract anomalies and oral-facial clefts (Wilson 2015). Although vitamin B12 and folate deficiencies are risk factors of NTD's, accumulating experimental evidence argues against a simple folate-deficiency model (Puvirajesinghe & Borg 2015).

Hyperhomocysteinemia is a medical condition characterised by exceedingly high concentrations of homocysteine (Hcy) in the blood, conventionally described above 15 µmole/L. Increased blood levels of Hcy result from an abnormal metabolic cycle (Greene et al. 2009; Rawls & Fisher 2010). Defective carbon metabolism can be caused by genetic mutation or nutritional deficiency in cofactors such as folate and vitamin B12. By-product build up occurs in the form of Hcy (Botto et al. 1999; Mobasheri et al. 2010; Puvirajesinghe & Borg 2015; Wilson 2015). Increased maternal levels of Hcy have been reported to elevate the probability for NTD development (Botto et al. 1999; Mobasheri et al. 2010; Puvirajesinghe & Borg 2015; Wilson 2015).

Genetic considerations for NTD's include gene polymorphisms that affect the efficiency of folate metabolism, effects of epigenetics or DNA methylation and associated chromosomal anomalies (Greene et al. 2009; Puvirajesinghe & Borg 2015; Wilson 2015) Chromosomal anomalies that result in NTD's include trisomy 18 and trisomy 3q (Lurie 2016; Rosa, Trevisan, Rosa, Lorenzen, Zen, Oliveira, Graziadio & Paskulin 2013).

A study conducted by Myrianthopoulos & Melnick (1987) postulated that variant mutations influencing NTD formation shows familial aggregation, however, does not follow the pattern of simple Mendelian inheritance (Myrianthopoulos & Melnick 1987). Greene et al. (2013) described NTD's as sporadic, with recurrence fitting an oligo-genic or multifactorial polygenic pattern, rather than simple dominant or recessive inheritance patterns with reduced penetrance.

Extracellular ligands –such as Bone Morphogenic Proteins (BMP's) -in the human body are key regulators in the functioning and patterning of the neural tube. Studies conducted by Bauer et al. (2002) revealed that a variant C1064A missense mutation of a BMP antagonist protein called *Noggin* is present in the blood line of NTD sufferers. Close relatives of NTD sufferers had the missense mutation without any developmental consequences. The study concluded that if the heterogeneous presence of the variant is causative of NTD's, the influence of the mutation is small. The precise aetiology of NTD's, therefore, remains uncertain (Bauer et al. 2002; McMahon et al. 1998) In addition, the embryology of the clinical variation of NTD's is poorly understood.

Clinical diagnosis of NTD's involves high resolution ultra-sound screening for foetal abnormalities in the second trimester of pregnancy (Greene & Copp 2009; Paraskevas, Tzika & Kitsoulis 2013; Racusin, Villarreal, Antony, Harris, Mastorbattistia, Shamshiraz, Belfort & Aagaard 2015; Wilson 2014). Highly efficient, however dangerous and invasive, procedures include amniocentesis. The biochemical composition of amniotic fluid changes between the various stages of gestation. Physiological and pathological changes in the mother and foetus can be monitored by the amniotic fluid (Puvirajesinghe & Borg 2015; Wilson 2014).

1.4.2. Spondylolysis

Spondylolysis is a common congenital defect caused by the unsuccessful fusion of the pars interarticularis (Figure 1.4-3) of the neural arch. Spondylolysis may also result from the fracture of the pars interarticularis, separating the vertebral body from the neural arch (Attiah et al. 2014; Kim & Laor 2010; Marawar 2014; Masnicová & Beňuš 2003; McAnany, Cho, Qureshi & Hecht 2014; Metkar et al. 2014; Peer & Fascione 2007; Wright et al. 2013). In reviewed literature, there is much controversy regarding the aetiology of spondylolysis (Alton, Patel, Lee & Chapman 2014; Attiah et al. 2014; Kim & Laor 2010; McAnany et al. 2014; Wright et al. 2013).

Spondylolysis and spondylolisthesis are common causes of lower back pain (Attiah et al. 2014; Metkar et al. 2014; Wright et al. 2013). Spondylolisthesis refers to anterior displacement of one vertebra relative to another (Attiah et al. 2014; Rustagi, Lavelle & Tallarico 2014). This was first defined by Herbinaux in 1782 (Attiah et al. 2014). Spondylolysis at L5 is the most common cause of spondylolisthesis (Attiah et al. 2014; Overley, McAnany, Andelman, Kim, Cho, Qureshi & Hecht 2016; Rustagi et al. 2014). Spondylolysis does, however, not necessarily lead to spondylolisthesis (Attiah et al. 2014; Wright et al. 2013).

Spondylolisthesis are most often observed at the lumbosacral junction. Compression of the L5 and sacral nerve roots may result in neurological deficit (Attiah et al. 2014; Wright et al. 2013). Colloca et al. (2012) states that disorders, such as spondylolysis, in the vertebral column contribute immensely to musculoskeletal pain in patients. It is hypothesised that musculoskeletal pain is caused by afferent nerve fibres of nocireceptors in the innervated spinal tissue caused by abnormal mechanics in the spine (Colloca et al. 2012).

Dysplastic spondylopathic conditions involve congenital malformation of the pars interarticularis (Peer & Fascione 2007). According to Kim & Laor (2010), a study conducted by Haukipuo et al. (1978), presented sufficient data to indicate the autosomal dominant inheritance with variable expressivity of a spondylolysis gene. Congenital theories implicate failure of fusion resulting in defects of the posterior arch, absent pedicles and articular pillar (Alton et al. 2014). Some authors suggest that specific sport activities result in a stress fracture of the pars interarticularis. Activities that increase probability of spondylolysis involve repetitive rotation and hyperextension of the lumbar spine (Alton et al. 2014; Attiah et al. 2014; Masnicová & Beňuš 2003; McAnany et al. 2014; Metkar et al. 2014; Peer & Fascione 2007). Traumatic theories implicated either a single traumatic event of great magnitude (Alton et al. 2014; Peer & Fascione 2007) or repetitive micro-trauma leading to a stress fracture (Alton et al. 2014).

There are several criteria to differentiate traumatic from congenital pathology. Spondylolysis is classified as traumatic if: (1) the size of the of the separation in the pars interarticularis is greater than three mm, shows mal-alignment of spinous processes and rotates when body masses are superimposed; (2) when the articular mass is anteriorly displaced by the fracture; (3) the fracture of the articular mass is not smoothly corticated; (4) acute fractures are characterised by oedema in surrounding soft tissue with accompanying neurologic changes;

and (5) dysplastic changes will not be present in ipsi-lateral pedicel and laminae (Alton et al. 2014).

Spondylolysis is subdivided into five categories: dysplastic, isthmic, degenerative, traumatic and pathogenic (Peer & Fascione 2007; Rustagi et al. 2014; Wright et al. 2013). Isthmic spondylopathic conditions involve lesions on the pars interarticularis resulting from stress on the vertebral column over time (Peer & Fascione 2007). Degeneration of the intervertebral disc can result in spondylothesis due to segmental instability and alterations of the articular processes (Peer & Fascione 2007; Rustagi et al. 2014). Finally, pathological spondylolysis results from subsequent complications associated with bone tumour or infection (Peer & Fascione 2007).

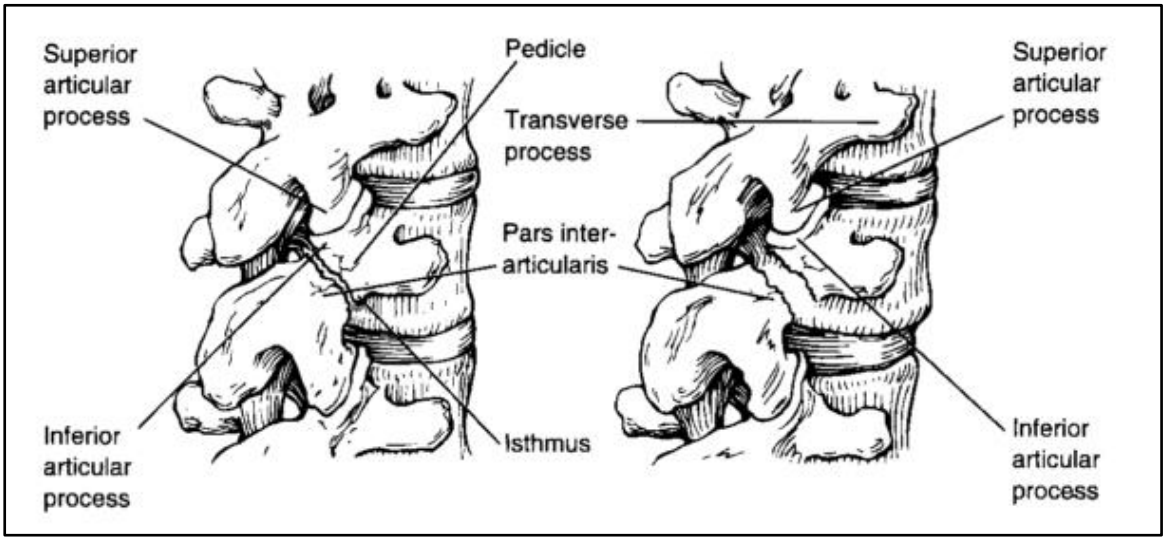


Figure 1.4-3: Depiction of Spondylolysis (Peer & Fascione 2007)

Spondylolysis in cervical vertebrae is very rare (Alton et al. 2014; McAnany et al. 2014). Only 100 (Alton et al. 2014) to a 150 (McAnany et al. 2014) cases have been reported in literature. Cervical spondylolysis (CS) is characterised by the disruption of the articular mass at the superior and inferior facet joints. Congenital CS is most commonly found at C6 (Kim & Laor 2010; McAnany et al. 2014).

The most feared complication of CS is injury to the spinal cord resulting in paralysis (Alton et al. 2014). Cord compression of CS is characterised by: (1) synovial proliferation in a neoarticulation; (2) hypertrophy of the articular process protruding into the spinal canal; (3) slip of the listhesis causing tetraplegia and (4) laminar instability with hypertrophy of the ligamentum flavum (Alton et al. 2014).

Spondylolysis is often asymptomatic and is diagnosed by chance during routine evaluation There are several treatment options for spondylolysis including management and surgical repair (Attiah et al. 2014; Lee, Ryu, Kim, Ahn, Kim & Yeom 2015; Menga, Jain, Kebaish, Zimmerman & Sponseller 2013; Metkar et al. 2014; Oishi, Sodeyama & Yanagisawa 2016; Overley et al. 2016; Peer & Fascione 2007; Rustagi et al. 2014; Wright et al. 2013).

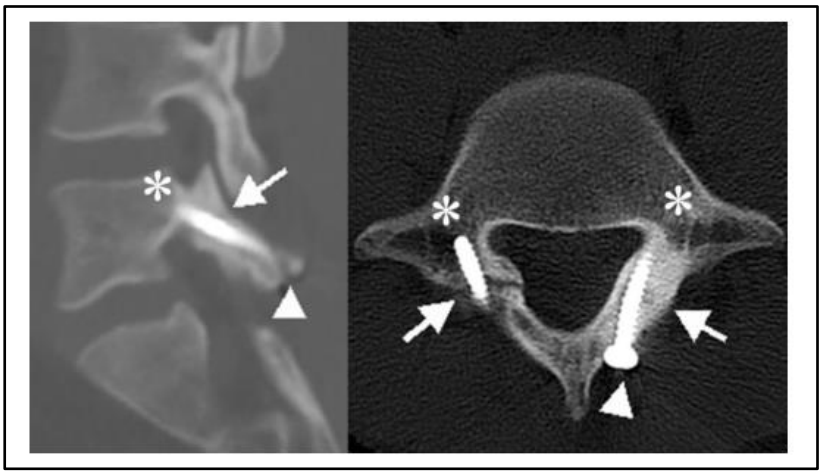


Figure 1.4-4: Surgical screw repair of pars interarticularis of vertebra (Lee et al. 2015)

A researched surgical treatment for lumbar spondylolysis is pars-screw fixation at L3, L4 and L5 vertebrae (Figure 1.4-4)(Lee et al. 2015; Menga et al. 2013).

1.4.3. Cranial-caudal border shifting

Variability in the vertebral column may arise when there is a shift from the typical distribution of vertebral segments in a region. This may cause an anomalous total number of vertebrae (Thawait et al. 2012). Cranial-caudal shifts of the vertebral column can be systemic or regional (Tague 2011b). Deviation from typical vertebral anatomy can result in confusion that leads to significant clinical errors (Thawait et al. 2012).

Bateson (1894), states that changes in vertebral counts are homeotic when ‘one of the component parts of the axial skeleton assumes the morphological appearance and function of its neighbour either immediately preceding or immediately following it… in distinction from meristic variations characterised by changes in total number of component parts’. This means that when an individual has variation in the vertebral column, it may result from (1) the addition of a segment known as meristic or (2) the change of one series identity at the expense of another known as homeotic (Asher et al. 2011).

A study conducted by Merbs (1974) observed that new world aborigines in the northern hemisphere tend to exhibit caudal shifts in the vertebral column (Tague 2011b).

1.4.3.1.Transitional vertebrae

Transitional vertebrae are abnormal vertebrae that are caused by overlapping of developing fields. The affected vertebra is intermediary, with combined anatomical morphology of the two adjacent vertebral regions (Chang, Park, Kyeong, Suk, Hae, Baek & Jung 2007; Khairnar & Rajale 2013; Konin & Walz 2010; Nakajima, Usui, Hosokai, Kawasumi, Abiko, Funayama & Saito 2014; Sekharappa, Amritanand, Krishnan & David 2014; Savage 2005).

Transitional vertebrae are common congenital anomalies in the lumbosacral region. The prevalence of LSTV has been reported to range between 3-32% (Chang et al. 2007; Kershenovich et al. 2015; Konin & Walz 2010; Sekharappa et al. 2014).

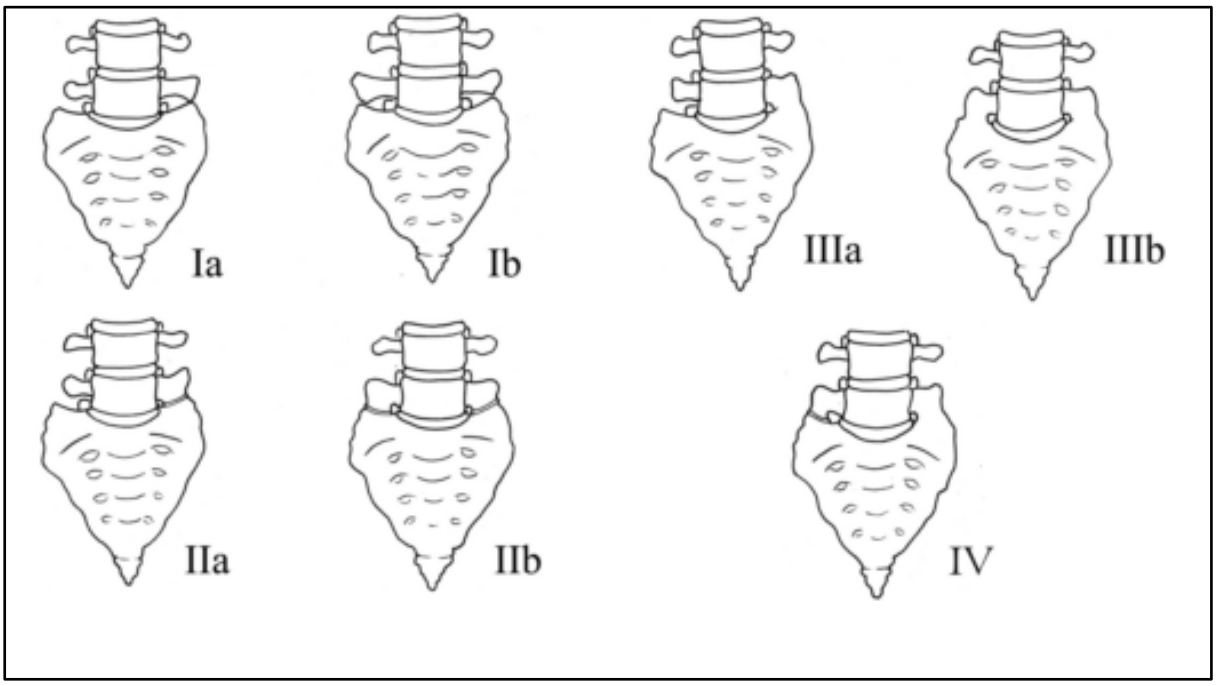


Figure 1.4-5: Classification of LSTV (Konin & Walz 2010)

Developmental defects at the lumbosacral border result in transitional vertebrae that have both lumbar and sacral anatomical features. A wide variety of configurations results from the defect (Figure 1.4-5) and is collectively referred to as LSTV. Morphological classification, according to Castellvi (1982), has identified four types of variant LSTV's. Type I LSTV's have a dysplastic large and triangular shaped transverse process observed unilaterally without fusion (Ia) or bilaterally, (Ib). Type II LSTV's are characterised by incomplete lumbarisation or incomplete sacralisation that may be unilateral (IIa) or bilateral (IIb). A diarthodial joint is

present in type II LSTV between itself and the sacrum. Type III LSTV's are characterised by complete lumbarisation or sacralisation that may be unilateral (IIIa) or bilateral (IIIb). In type III LSTV there is complete osseous fusion between the transitional vertebrae and the sacrum. Type IV LSTV have combined incomplete and complete features (Carrino et al. 2011; Konin & Walz 2010; Metkar et al. 2014; Paraskevas et al. 2013; Samreen, Shashikala & Rohini 2012; Sekharappa et al. 2014; Uçar, Uçar, Bulut, Azboy & Demirtaş 2013).

According to Barnes (1994), LSTV are caused by a delay of the timing threshold event in the lumbosacral regions which postulates that the developmental field expand beyond the normal parameters resulting in boundary shift at the transitional areas of the vertebral column (Savage 2005).

Literature states that a caudal shift at the lumbosacral junction results in lumbarisation; defined as the non-fusion of the first sacral segments. Contrarily, cranial shifts result in sacralisation; defined as fusion of the distal lumbar segment to the sacrum. In addition, the direction of the shift may result in either augmented or diminished numbers of lumbar or sacral segments (Chang et al. 2007; Mahato 2010; Nakajima et al. 2014; Savage 2005; Tague 2011b; Uçar et al. 2013). Studies have reported that LSTV can be identified by all imaging modalities (Chang et al. 2007; Konin & Walz 2010; Sekharappa et al. 2014).

Based on the biomechanical changes in the vertebral column, several researchers theorise that Bertolloti's syndrome is associated with LSTV and back pain. There is, however, controversy regarding this theory, as the exact relationship is unknown (Chang et al. 2007; Khairnar & Rajale 2013; Mahato 2010; Savage 2005; Sekharappa et al. 2014; Uçar et al. 2013; Barnes 2012). Some researchers stipulate that LSTV has no clinical impact on patients (Uçar et al. 2013; Sekharappa et al. 2014). Other researchers contradict this statement and state that LSTV may predispose patients to certain clinical disorders (Uçar et al. 2013).

The clinical relevance of LSTV was reported to be significant in some aspects. A case report published by Paraskevas et al. (2013) associated a single case of spina bifida occulata to LSTV. In addition, a study conducted by Khairnar et al. (2013), concluded that the intervertebral discs are significantly narrower in patients with LSTV and that an increased predisposition to spondylolisthesis was observed. The findings regarding intervertebral disc pathology was corroborated by a study conducted by Sekharappa et al. (2014). The study stipulated that a definite association was noted between LSTV and intervertebral disc degeneration (Sekharappa et al. 2014). The significant importance of the variation was

emphasised in the clinical practice of surgeons, radiologists, physiotherapists and anaesthesiologists (Khairnar & Rajale 2013; Paraskevas et al. 2013).

Transitional vertebrae retain partial features of the adjacent regions. Very little is known about transitional vertebrae at the thoracolumbar junction. According to Thawait et al (2012), TLTV in the thoracic region were defined by Wigh as the presence of hypoplastic ribs that are less that 3.8 cm in length on the lowest rib bearing segment. In addition, the author states that the prevalence of TLTV is unknown (Thawait et al. 2012; Carrino et al. 2011).

1.4.3.2.Sacro-coccygeal fusion

The sacro-coccygeal joint is a mobile synovial type joint between the sacrum and the coccyx (Drake et al. 2009; Moore et al. 2010; Tague 2011b). In some cases sacro-coccygeal fusion may occur. Fusion of the coccyx to the sacrum results from caudal shift of the vertebral column. Tague (2011a) states that the reported prevalence in literature of sacro-coccygeal fusion ranges between 0-71.7%. The reason for the high variance in prevalence remains unknown. It has been observed that sacro-coccygeal fusion is more prevalent in males and increases in prevalence with age (Tague 2011b).

There is much controversy among published literature regarding the clinical significance of sacro-coccygeal fusion. Some authors state that the only effect of coccygeal fusion to the sacrum is to increase the length of the sacrum (Tague 2011b). Other authors state that sacro-coccygeal fusion has clinical application in obstetrics. Gueriero et al. (1940) states that a "…prominent coccyx with anterior angulation and ankylosis at the sacro-coccygeal articulation…may hinder natural delivery".

1.5.PROBLEM STATEMENT

In reviewed literature, case studies have reported various congenital defects that are simultaneously present within the vertebral column of an individual. There is, however, a lack of evidence to substantiate whether the mutually inclusive observations resulted by chance, or whether an association between the defects are present. The exact relationship between the defects remains unknown. Many publications are available to discuss the typical development of the vertebral column in humans. There still, however, exists much controversy regarding the mechanisms that result in defects in the vertebral column.

Lastly, several publications discuss the characteristics and classification of transitional vertebrae at the lumbosacral junction (LSTV). Despite the abundant literature on LSTV, little

information has been published about transitional vertebrae at the thoracolumbar junction (TLTV). Several theorists suggest theories regarding the etiology and clinical implication of transitional vertebrae. The exact mechanism and clinical relevance, however, has remained uncertain.

The aim of this study was to identify random congenital defects in the vertebral column that result from defective neural arch formation or cranial-caudal shifts and evaluate whether associations exists among them. In addition, this study aimed to identify TLTV based on intermediary characteristics between the thoracic and lumbar regions present in the vertebrae.

This study hypothesised that there is an association between random congenital defects in the vertebral column that result from cranial-caudal border shift or defective neural arch formation.

MATERIALS AND METHODS

2.1.MATERIALS

In this study, skeletal material from the Kirsten Skeletal Collection at Stellenbosch University, Tygerberg Medical Campus was evaluated. The skeletal remains of all individuals within the collection (N=±1100) functioned as the selected population of this study. A selection of specimens (n=35) were taken from a subset with random congenital defects in the vertebral column.

Instruments used to record the qualitative data in this study, was an electro-optical system (EOS) Digital Canon Camera. Qualitative data sources include observations using interpretive techniques.

2.2.METHODS

2.2.1. Methodology

The purpose of research is to discover answers to questions through the application of scientific procedures. The purpose of the research determines the methodology used (Kothari 2004).

This was a diagnostic research study. Diagnostic research studies determine the frequency with which a characteristic occurs or determine the association between the various characteristics. This type of research methodology uses qualitative data (Kothari 2004). Qualitative study methodology provides tools for researchers to study complex phenomena. It is a valuable method in health science research to develop theory and prospective interventions. Conversely, quantitative data is centred on the process of measurements and mathematical expression of numerical relationships (Kothari 2004; Page & Page 2013). The purpose of this study was to determine the frequency of random congenital defects, within a set boundary of defects, in the vertebral column. This was, subsequently, used to evaluate whether associations are present between random congenital defects in the vertebral column in a qualitative manner. Prospective research will include quantitative research methodology for future clinical application reference to findings in this study.

2.2.2. Criteria for selection

A selection of specimens (n=35) was chosen from a subset of specimens with random congenital defects in the vertebral column. It was required for all specimens in the selection to have: (1) no discernible post-mortem damage of vertebrae, (2) accountability of all

vertebrae and (3) at least one random congenital defect in the neural arch of the vertebral column. Any specimen that adhered to the above stated requirements was included in the study.

The defects considered in this study comprised the most common skeletal defects in the vertebral column (Masnicová & Beňuš 2003). The congenital defects of the vertebral column included in the study were: NTD in vertebrae, spondylolysis, cranial-caudal border shifts, sacralisation of coccygeal vertebrae and transitional vertebrae at the thoracolumbar or lumbosacral junctions. Subtypes of LSTV were classified according to Castellvi (1982).

2.2.3. Aims

The aim of this study was to identify random congenital defects in the vertebral column that result from defective neural arch formation or cranial-caudal shifts and evaluate whether associations exists among them.

In addition, this study aimed to identify TLTV based on intermediary characteristics between the thoracic and lumbar regions present in the vertebrae.

2.2.4. Hypotheses

There is an association between random congenital defects in the vertebral column that result from cranial-caudal border shift or defective neural arch formation.

2.2.5. Data collection

There is an abundance of published literature to provide a control for qualitative observations in normal vertebrae (Drake et al. 2009; Hansen 2010; Moore et al. 2010; Netter 2011). A diagnostic research study was conducted to obtain qualitative information about the frequencies of random congenital defects within each vertebral column from the selection. Data was collected from each specimen in the selection (n=35).

The vertebrae for each vertebral column were sequenced in to the correct order. Numbering of the vertebrae was completed according to Bron et al. (2007). This method stipulates that numbering of vertebrae in the vertebral column is assigned relative to rib articulation. The last vertebra with costal articulation is numbered as the last thoracic vertebra, as the functional region of the vertebra is the thoracic region. The next caudal vertebra is numbered as the first lumbar vertebra (Sekharappa et al. 2014).

The sequence of observations included (1) tabulating specific observations and (2) taking photographs of the vertebrae.

2.2.6. Data analyses

The statistic techniques applied to different sets of data is dictated by the nature of the data (Freund 2011). This simply implies that quantitative and qualitative data are collected and characterised differently. Qualitative research is concerned with understanding complex phenomena (Page & Page 2013).

Statistical analysis was performed using the following programme: © Microsoft Excel XLStat extension pack. This programme is a free © Microsoft Excel hold-on that can be used to analyse both qualitative and quantitative data sets. Further statistical consultation was provided by Dr Jana Jacobs.

The frequencies of congenital defects in the vertebral column were listed in qualitative frequency charts. Frequency distributions are a graphical presentation of untreated data in a broad compact manner.

Graphical presentations, however, are a more effective manner to illustrate a large magnitude of information (Freund 2011). In this study, stacked bar graphs were constructed to illustrate the frequencies of congenital defects in the vertebral column from the frequency distributions. This method effectively demonstrates the frequencies of defects in specimens and the overlap of defects observed in a specimen.

Data analysis of qualitative data in a diagnostic research study describes only in detail the frequencies of observations of characteristics to discuss possible associations between the characteristics (Kothari 2004). The data in this study was analysed by providing a detailed description of the frequencies and associations, based on corresponding qualitative observations, were described.

2.2.7. Ethics

This study was ethically cleared by the Health Research and Ethics Committee of Stellenbosch University which conforms to the principles within the Declaration of Helsinki (1964).The allocated ethics number is S13/05/100.

2.2.8. Limitations

The selection of skeletal remains used in the study was confined to the population of the Kirsten Skeletal Collection at Stellenbosch University (section 2.1).

The subset of individuals with congenital defects in the population is limited. The subset selection of individuals in a population will, therefore, be confined.

Post-mortem loss and damage of vertebrae within a specimen further diminished the number of specimens that were viable for the study within the population.

The congenital defects were exclusive to a select set of defects in the vertebral column that resulted from defective neural arch formation and cranial-caudal border shifts. Congenital defects of the vertebral body were not considered in this study. This limitation did not influence significant observations noted in the study. It was, however, a necessary exclusion criterion that provided structure to the study.

It is impossible to accurately re-construct the specific past embryological events that occurred in the individuals from the selection, which resulted in the defects observed.

RESULTS

3.1.OVERVIEW

Descriptive analysis was used to find the frequencies of defects in the selection. The descriptive analyses are illustrated in frequency distribution tables for each defect evaluated in the study. General overview of the frequencies for congenital defect types is illustrated in Table 3.1-1.

Table 3.1-1: Frequency distribution of specimens with congenital defects

Category	Frequency	Relative Frequency	Frequency Percentage
Spondylolysis	8	0,2286	22,86%
NTD	17	0,4857	48,57%
LSTV	14	0,4000	40,00%
TLTV	35	1,0000	100,00%
Sacro-coccygeal fusion	5	0,1429	14,29%

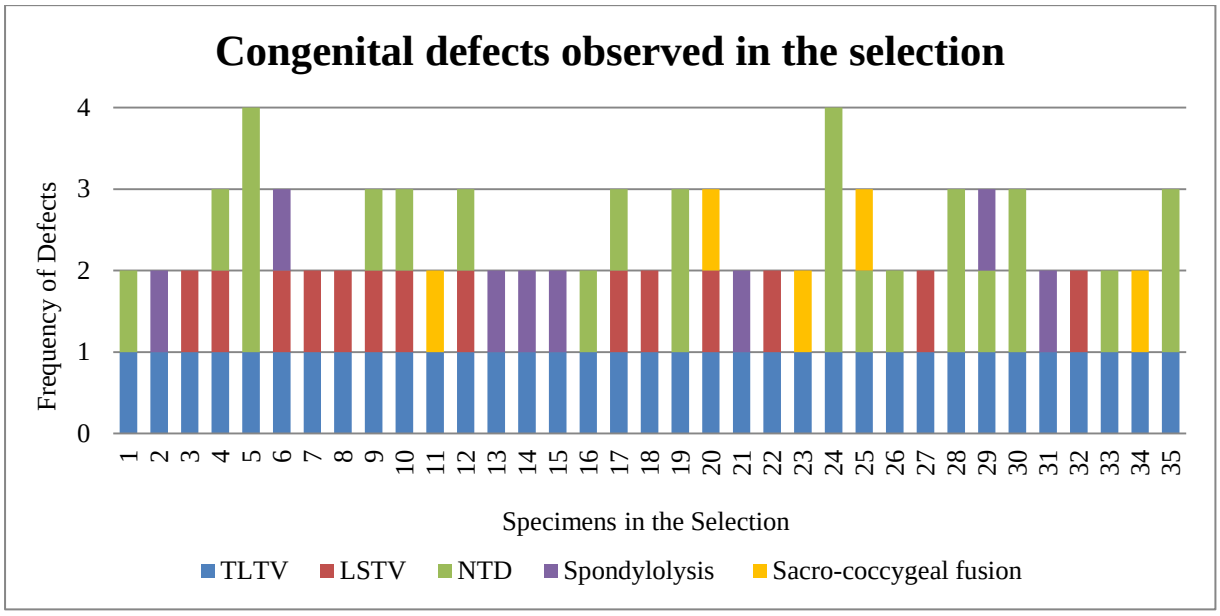


Figure 3.1-1: Stacked bar graph illustrating observed congenital defects

It was observed that specimens in the selection had overlapping anomalies in the vertebral column (Figure 3.1-1). It was, therefore, required to assess each defect separately and check for associations among the various defects that were present within the selection. In addition, more profound analysis was required to illustrate the subtypes of each defect present within the selection.

3.2.SACRO-COCCYGEAL FUSION

The subset of specimens with sacro-coccygeal fusion from the selection was evaluated. Fusion of the sacrum and coccyx was observed in 14.29% ($f = 5$) of the selection. Descriptive evaluation was used to determine whether associations are present between sacro-coccygeal fusion and other congenital defects observed in the selection.

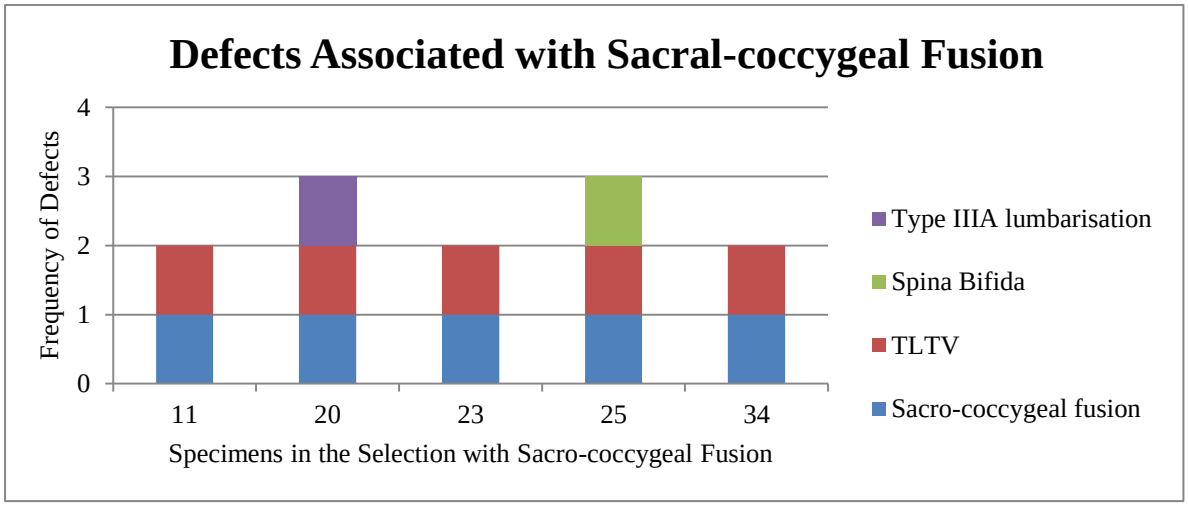


Figure 3.2-1: Defects associated with sacro-coccygeal fusion

Every specimen in the selection with sacro-coccygeal fusion ($f = 5$) exhibited features of TLTV (Figure 3.2-1). Spina bifida was associated with sacro-coccygeal fusion and TLTV in one specimen ($f = 1$) from the selection (Figure 3.2-1). Lastly, one specimen ($f = 1$) from the subset in the selection with sacro-coccygeal fusion had spina bifida in addition to TLTV (Figure 3.2-1). No associations between other defects considered in this study and sacro-coccygeal fusion were observed.

3.3.SPONDYLOLYSIS

In the subset of specimens with spondylolysis from the selection, analysis was conducted to evaluate associations present between spondylolysis and other anomalies considered in the study. Spondylolysis were present in 22.86% ($f = 8$) of the selection (Table 3.1-1).

Every specimen in the selection with spondylolysis ($f = 8$) had TLTV present at the thoracolumbar junction (Figure 3.3-1). Spondylolysis and type IIa LSTV were associated in one specimen ($f = 1$) from the selection (Figure 3.3-1). Lastly, spondylolysis was present with spina bifida in one specimen ($f = 1$) from the selection (Figure 3.3-1).

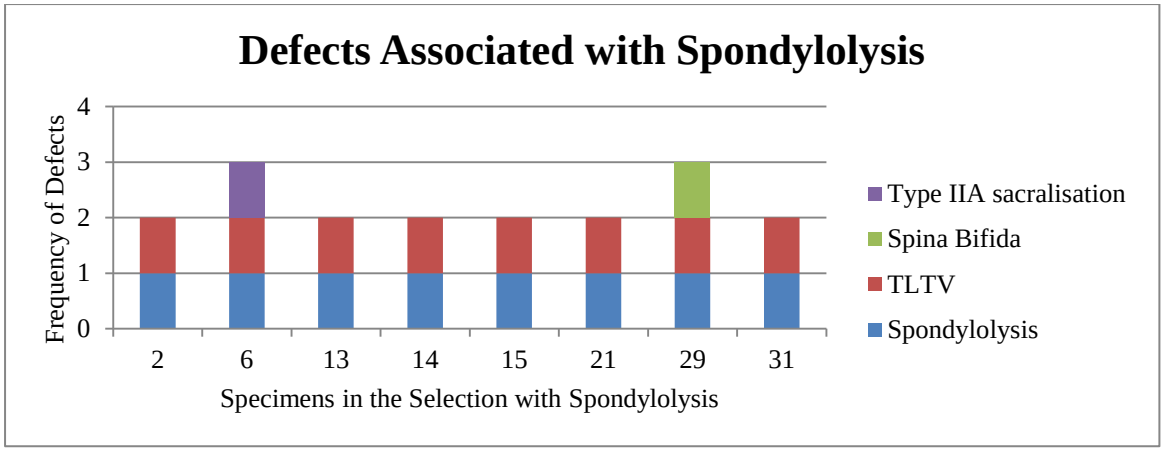


Figure 3.3-1: Defects associated with spondylolysis in cases from the selection

None of the cases, from the subset of specimens with spondylolysis, presented association with sacro-coccygeal fusion. No other associations between spondylolysis and other defects included in the study were observed in any of the cases.

3.4.LSTV

The subset of specimens with LSTV was evaluated. In total, 40 % ($f = 14$) of the selection had specimens with LSTV (Table 3.1-1). Further evaluation was conducted to find associations present between LSTV and other defects observed in the study.

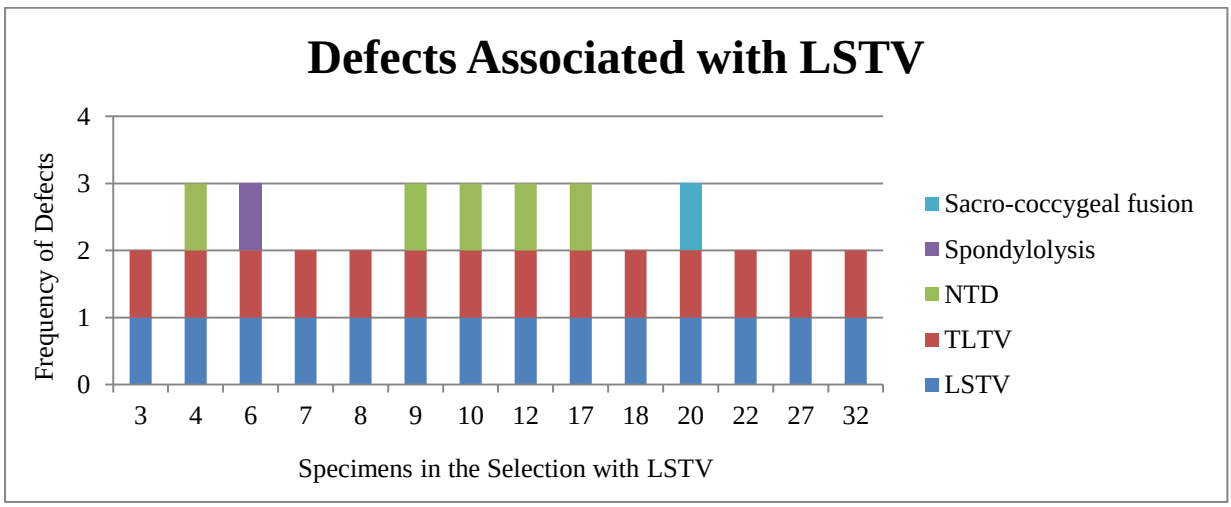


Figure 3.4-1: Defects associated with LSTV in cases from the selection

Every specimen in the selection with LSTV ($f = 14$) exhibited features of TLTV (Figure 3.4-1). Sacro-coccygeal and LSTV were associated with one another in one specimen ($f = 1$) from the selection. In addition, one specimen ($f = 1$) from the selection presented

spondylolysis and LSTV. Lastly, NTD's were associated with LSTV in five ($f = 5$) specimens from the selection (Figure 3.4-1).

A more detailed evaluation was required to show the frequencies of classified LSTV's in the selection. Lumbosacral transitional vertebrae are broadly classified into lumbarisation and sacralisation. Sub-classifications are provided according to Castelvi (1982) (discussed in 1.4.3.1).

Table 3.4-1: Frequency distribution of classified LSTV

Category	**Frequency**	**Relative Frequency**	**Frequency Percentage**
LSTV categories			
Type Ia sacralisation	2	0,0571	5,71%
Type IIa sacralisation	3	0,0857	8,57%
Type IIIb sacralisation	5	0,1429	14,29%
Type IIIa sacralistion	1	0,0286	2,86%
Type IV sacralisation	1	0,0286	2,86%
Type IIIa lumbarisation	2	0,0571	5,71%
Total Lumbarisaiton	*2*	*0,0571*	*5,71%*
Total Sacralisation	*12*	*0,3429*	*34,29%*
Total LSTV	**14**	**0,4000**	**40,00%**

Sacralisation of L5 was present in 34.29% ($f = 12$) specimens within the selection (Table 3.4-1). It was observed that type Ia sacralisation was present in 5.71% ($f = 2$) of the cases (Table 3.4-1). Type IIa sacralisation was present in 8.57% ($f = 3$) of the specimens (Table 3.4-1). One specimen showed features of type IIIa sacralisation (Table 3.4-1). Type IIIb sacralisation was observed in 14.29% ($f = 5$) of the specimens in the selection. Type IV sacralisation was present in one ($f = 1$) of case (Table 3.4-1).

Lumbarisation was present in 5.71% ($f = 2$) of the selection. Both specimens exhibited features characterised as type IIIa lumbarisation of S1 (Table 3.4-1).

3.5.NTD'S

NTD's were restricted to incomplete posterior neuropore fusion of the vertebral column. The NTD's observed in the vertebral column included bifurcations or clefts in the neural arch and spina bifida in the sacrum.

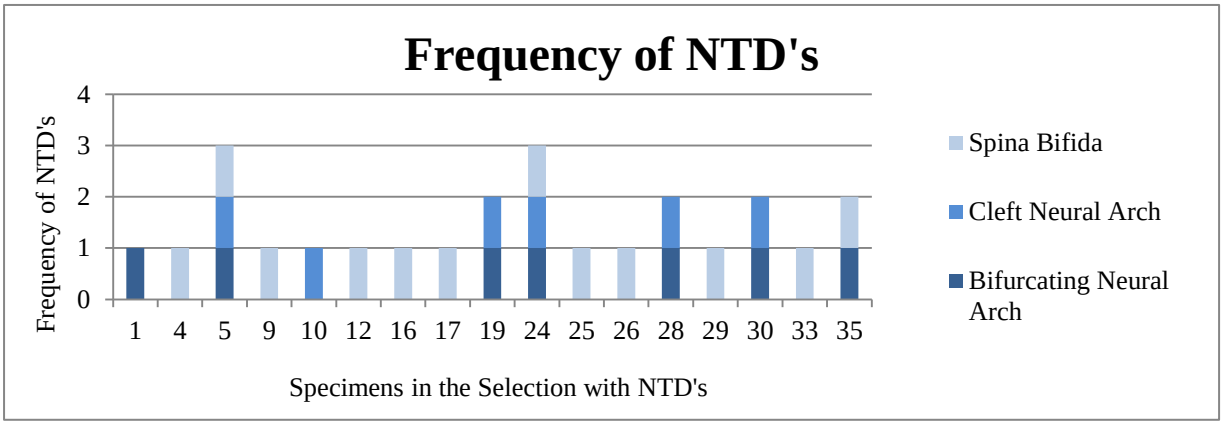


Figure 3.5-1: Illustration of various NTD's observed in specimens from the selection

Table 3.5-1: Frequency distribution of different NTD's in the selection

Category	**Frequency**	**Relative Frequency**	**Frequency Percentage**
NTD categories			
Spina bifida	9	0,2571	25,71%
Bifurcating neural arch	1	0,0286	2,86%
Spina bifida and bifurcating neural arch	1	0,0286	2,86%
Cleft and bifurcating neural arch	3	0,0857	8,57%
Spina bifida, cleft and bifurcating neural arch	2	0,0571	5,71%
Cleft neural arch	1	0,0286	2,86%
Total specimens with spina bifida	*12*	*0,3429*	*34,29%*
Total specimens with cleft neural arches	*6*	*0,1714*	*17,14%*
Total specimens with bifurcating neural arches	*7*	*0,2000*	*20,00%*
Total specimens with NTD's	**17**	**0,4857**	**48,57%**

In the study, 48.57% ($f = 17$) of the selection had at least one random NTD present in the vertebral column (Table 3.1-1). The subset of specimens with NTD's in the vertebral column was further analysed. In the subset selection with random NTD's in the vertebral column, 25.71% ($f = 9$) (Table 3.5-1) of the specimens had spina bifida, with no other NTD in the

vertebral column (Figure 3.5-1). In addition, there was one specimen (2.86%) (Table 3.5-1) that had only a bifurcation in the posterior neuropore with no other observed NTD's (Figure 3.5-1). There were two specimens ($f = 2$) (Table 3.5-1) that had a combination of every NTD considered in this study (Figure 3.5-1). In addition, 8.57% ($f = 3$) of the specimens had both clefts and bifurcations in the neural arch present (Figure 3.5-1).

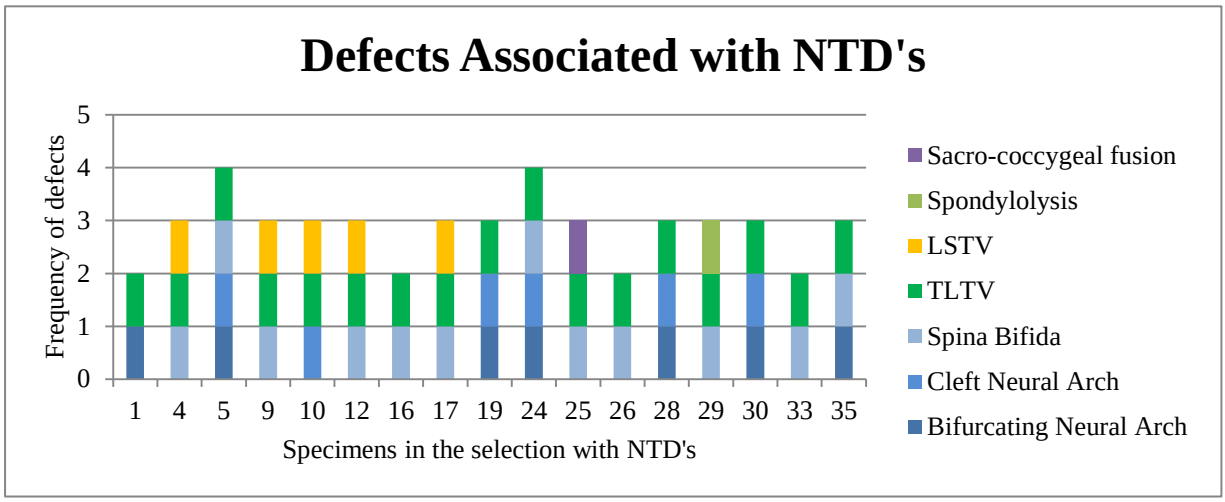


Figure 3.5-2: Defects associated with NTD's in specimens from the selection

The relationships between random NTD's in the vertebral column and other congenital defects in the vertebral column were evaluated. The results show that TLTV were present in every specimen from the selection with at least one random NTD in the vertebral column ($f = 16$)(Figure 3.5-2). Spondylolysis was association with NTD in only one case ($f = 1$) within the selection. In addition, it was observed that NTD's were associated with LSTV and TLTV in five ($f = 5$) of the specimens from the selection. Lastly, sacro-coccygeal fusion was associated with NTD's in one specimen ($f = 16$).

3.6.TLTV

Every specimen in the selection ($f = 35$) had TLTV (Table 3.1-1) (Figure 3.1-1). Identification of TLTV was based on intermediary thoracic and lumbar features observed in vertebrae at the thoracolumbar junction (T12-L1).

Every TLTV ($f = 35$) exhibited the following features: aplasia or hypoplasia of the transverse process, intermediary facet orientation on the superior articular process and intermediate mammillary bodies located between the superior articular process and transverse processes (Figure 3.6-1).

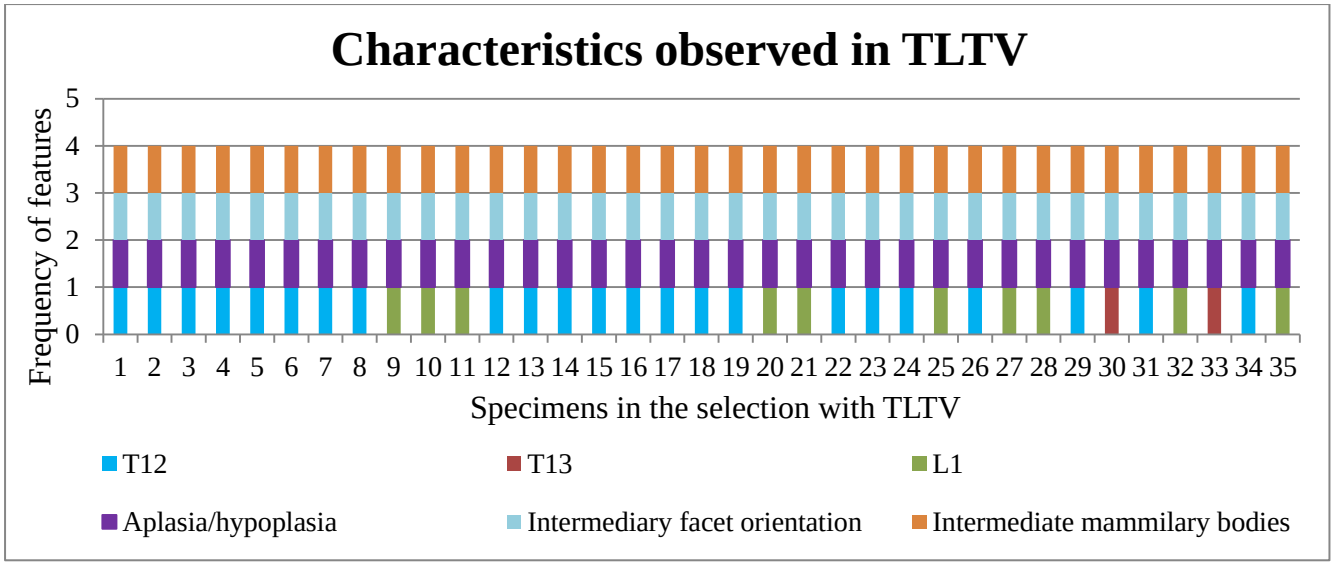


Figure 3.6-1: Observations of TLTV characteristics

Classification of TLTV was based on the location in the vertebral column. The frequency chart of TLTV shows that 65.71% ($f = 23$) of TLTV were located at T12 (Table 3.6-1). This location was the most frequent position of TLTV in the selection. The second most frequent location of TLTV in the vertebral column was L1. It was observed that 28.57% ($f = 10$) of the TLTV were located at L1 (Table 3.6-1). In two specimens of the selection 5.71% ($f = 2$), T13 were transitional of the thoracolumbar junction (Table 3.6-1).

Table 3.6-1: Frequency distribution of TLTV locations

Category	**Frequency**	**Relative Frequency**	**Frequency Percentage**
TLTV categories			
T12TLTV	23	0,6571	65,71%
T13TLTV	2	0,0571	5,71%
L1TLTV	10	0,2857	28,57%
Total specimens with TLTV	**35**	**1,0000**	**100,00%**

DISCUSSION

Skeletal remains of individuals can be assessed to study various anomalies in humans that affect bone. Publications have reported case studies of different congenital defects in the vertebral column that are present within an individual (Paraskevas et al. 2013). None of the publications have, however, been able to fully describe the mutual association among the defects of the vertebral column in other individuals.

In this study, skeletal remains of a select subset (n=35) of specimens with random congenital defects in the vertebral column were evaluated. The specimens were selected based on the requirement that at least one random congenital defect must be present in the vertebral column. The random congenital defects that were observed included: NTD's, LSTV, TLTV, spondylolysis and sacralisation of the coccyx.

4.1.TLTV

Unlike the other congenital defects evaluated in this study, very little is known about transitional vertebrae at the thoracolumbar junction. Literature states that transitional vertebrae at any junction are characterised by the intermediary features retained from the two adjacent regions in the vertebral column (Carrino et al. 2011; Chang et al. 2007; Khairnar & Rajale 2013; Konin & Walz 2010; Nakajima et al. 2014; Savage 2005; Sekharappa et al. 2014; Thawait et al. 2012).

Wigh (1980) identified TLTV by the presence of hypoplastic ribs that are less that 3.8 cm in length on the lowest rib bearing segment (Carrino et al. 2011; Thawait et al. 2012). This method only considers TLTV in the thoracic region; the presence of TLTV in the lumbar region is disregarded. The descriptive technique described by Wigh (1980) is based on features of ribs rather than characteristics of the vertebrae itself. This technique neglects to include other overlapping thoracic and lumbar features that the transitional vertebrae may possess. In addition, there is no classification system available for TLTV. This identification reference was, therefore, not used in this study. The most frequent congenital defect present in the vertebral column of the selection was TLTV. Transitional vertebrae at the thoracolumbar junction were identified based on intermediary features of adjacent thoracic and lumbar regions. The following observations were made about the intermediary characteristics of TLTV in this study.

Transitional vertebrae at the thoracolumbar junction are atypical vertebrae with many variations that are collectively referred to as TLTV. The morphological variations of TLTV

are intermediary of typical thoracic and lumbar regions; yet distinctly different from either region. The following general morphological traits were observed in TLTV.

Normal lumbar vertebrae have mammillary bodies on the superior articular processes (Figure 4.1-2). Conversely, this feature is not present in normal thoracic vertebrae (Figure 4.1-1). In TLTV, it was observed that intermediary or unilateral mammillary bodies located between the transverse and superior articular processes (Figure 4.1-3; Figure 4.1-4).

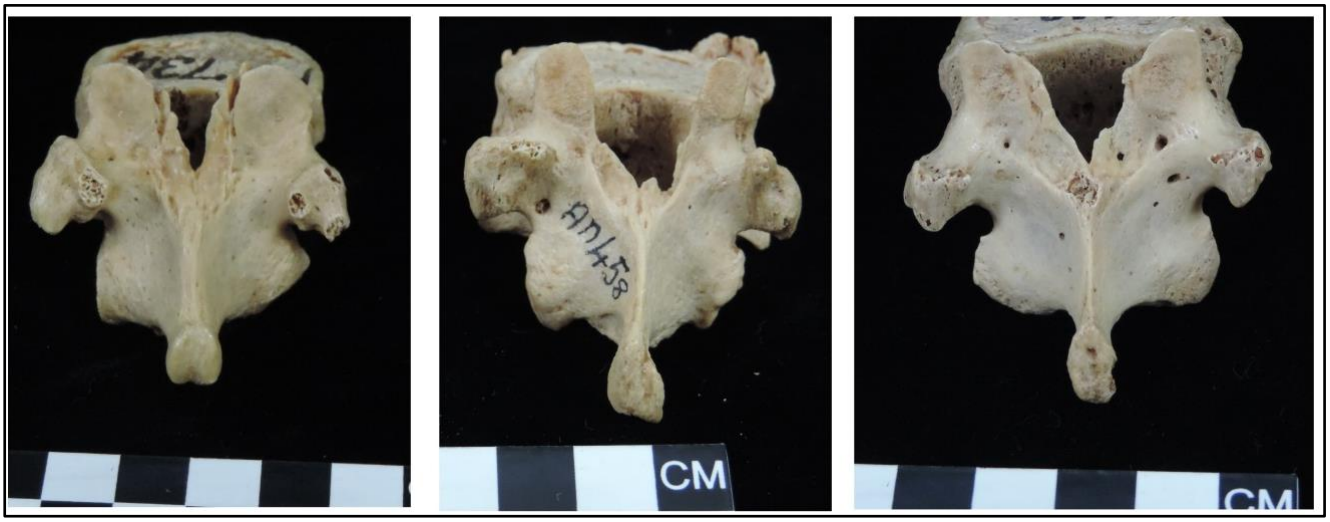


Figure 4.1-1: Normal thoracic vertebrae (T12)

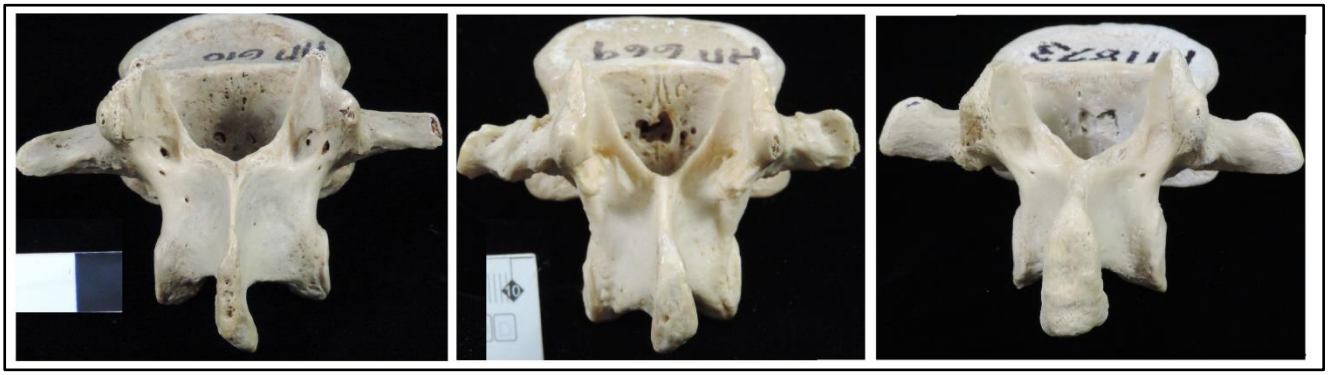
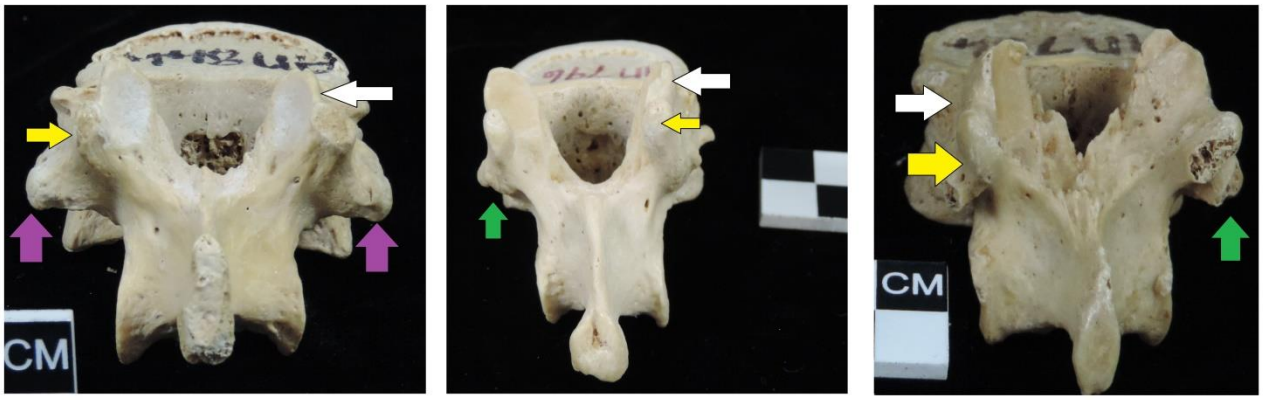


Figure 4.1-2: Normal lumbar vertebrae (L1)

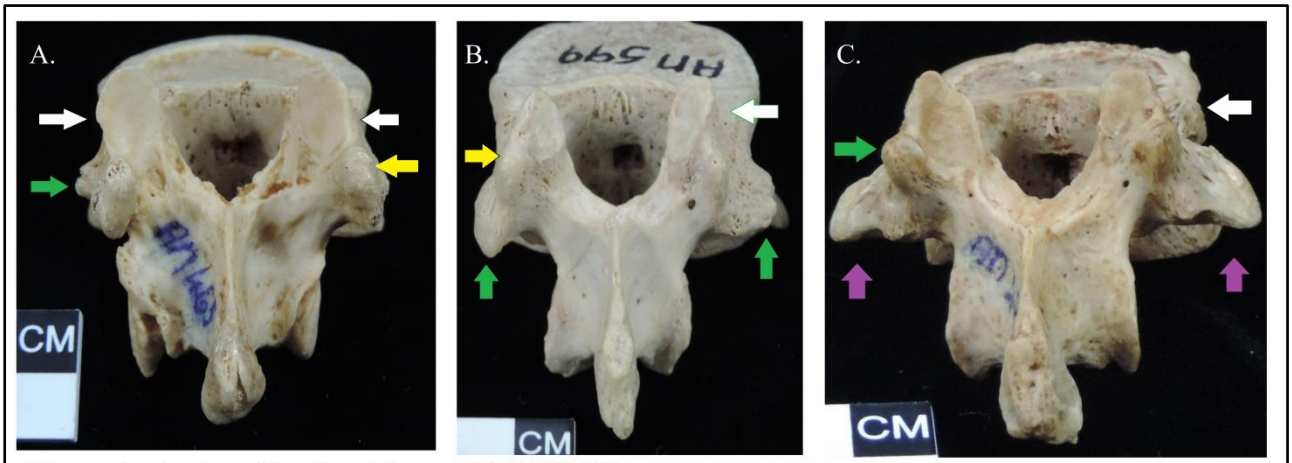
In normal thoracic vertebrae, the superior articular facets are directed posteriorly (Figure 4.1-1). Conversely, the superior articular facets in normal lumbar vertebrae are directed medially (Figure 4.1-2). This study observed that the superior articular facets of TLTV are orientated in directions that resemble both regions. This can be bilaterally where the angles lie posterior-medially or unilaterally where the facets are asymmetrical (Figure 4.1-3; Figure 4.1-4).



Thoracolumbar transitional vertebrae at T12 (T12TLTV)

1. Intermediate postero-medial orientation of superior articular facets (white arrows). Anomalous relative to the posterior orientation in normal thoracic vertebrae.
2. Intermediate mammilary bodies present, not characterised in normal thoracic vertebrae (yellow arrows)
3. Hypoplasia (purple arrows) or aplasia (green arrows) of transverse processes

Figure 4.1-3: Transitional vertebrae at T12 (T12TLTV)



Thoracolumbar transitional vertebrae at L1 (L1TLTV)

1. Asymetric orientation of superior facets. Not directed medially as observed in normal lumbar vertebrae (white arrows)
2. Intermediate mammilary bodies located between superior facets and transverse process (yellow arrows)
3. Agenesis (green arrows) or hypoplasia (purple arrows) of transverse processes

Figure 4.1-4: Transitional vertebrae at L1 (L1TLTV)

Vertebrae in the lumbar region are characterised by long slender transverse processes (Figure 4.1-2). Alternatively, vertebrae in the lower thoracic region have prominent short, stout transverse processes (Figure 4.1-1). In general, hypoplasia or aplasia of the transverse processes was observed in transitional vertebrae at the thoracolumbar junction (Figure 4.1-3; Figure 4.1-4).

This study observed that TLTV may be located in either the thoracic or lumbar regions of the vertebral column. According to Bron et al. (2012), vertebrae with costal facets for rib articulation are thoracic vertebrae. Contrarily, lumbar vertebrae do not have costal facets on the vertebral bodies. Transitional vertebrae can, therefore, be classified according to the functional region that it is located in.

Within the selection, majority of TLTV, 65.71% ($f = 23$), were located at T12. In these cases, the transitional vertebrae assume the position of T12 in the vertebral column. The remaining specimens in the selection ($f = 12$) had normal T12 vertebrae. Transitional vertebrae at T12 result from segmental shifts that affect the twelfth thoracic vertebrae with no additional segments present. Despite the resemblance of T_{12}TLTV to vertebrae in the lumbar region, the vertebrae have costal facets for rib articulation and are, therefore, classified as thoracic. When normal thoracic vertebrae (Figure 4.1-1) are compared to TLTV located at T12 (Figure 4.1-3) the variations in morphology can be seen. As is seen in Figure 4.1-3, the superior articular facets of T_{12}TLTV are not directed posteriorly as is seen in normal T12. Unlike T12, there are structures resembling mammillary bodies located between the superior articular and transverse processes. The most accurate manner in which to describe the structures are "intermediary" mammillary bodes. Lastly, T_{12}TLTV do not have prominent transverse processes, instead aplasia or hypoplasia of the transverse process is observed. At least one additional vertebral column defect was seen in specimens with T_{12}TLTV.

The less frequent location of TLTV is at L1, present in 28.57% ($f = 10$) of the selection. In cases such as these, the transitional vertebrae assume the position of L1. The remaining specimens in the selection ($f = 15$) showed normal L1 morphology. The L_1TLTV resembles vertebrae in the thoracic region, but does not have costal facets for rib articulation. When the features of lumbar vertebrae (Figure 4.1-2) are compared to L_1TLTV (Figure 4.1-4), the variation in morphology can be seen. As is seen in Figure 4.1-4, L_1TLTV do not have long slender transverse processes characteristic of the lumbar region. Instead, aplasia or hypoplasia of the transverse processes was observed. The superior facets of L_1TLTV are not orientated in a symmetrical medial direction, but are asymmetrical and more posteriorly. Lastly, L_1TLTV do not have typical mammillary bodies present; rather remnant features of mammillary bodies that are located between the superior articular and transverse processes. In every specimen with L_1TLTV, one additional congenital defect was observed in the vertebral column.

There are two types of L_1TLTV that was observed. The features of both L_1TLTV types remain the same. If TLTV located at L1 result from segmental shifts that alter the morphology of the first lumbar vertebrae, without any additional lumbar segments present, it was referred to as L_{1a}TLTV. Majority of L_1TLTV in the study ($f = 13$) were L_{1a}TLTV.

In rare cases it was observed that an individual developed an additional somite segment at the thoracolumbar junction. If the somite develops in the lumbar region, an additional lumbar vertebra will be present ($f = 2$). The additional somite (L_{1b}TLTV) forces a caudal shift of other somites and resumes the position of L1. The vertebral column will, therefore, be characterised by six lumbar vertebrae. The number of vertebrae in the other regions remains unchanged. There will be seven cervical, 12 thoracic and five sacral vertebrae in the vertebral column.

Alternatively, if the additional somite develops in the thoracic region, it will function as an additional vertebra in the thoracic region. The individual will, therefore, have 13 pairs of ribs correlating to 13 thoracic vertebrae. This study identified two specimens ($f = 2$) with 13 thoracic vertebrae in the vertebral column. The transitional vertebrae were T_{13}TLTV. The number of vertebrae in the other regions remains unchanged.

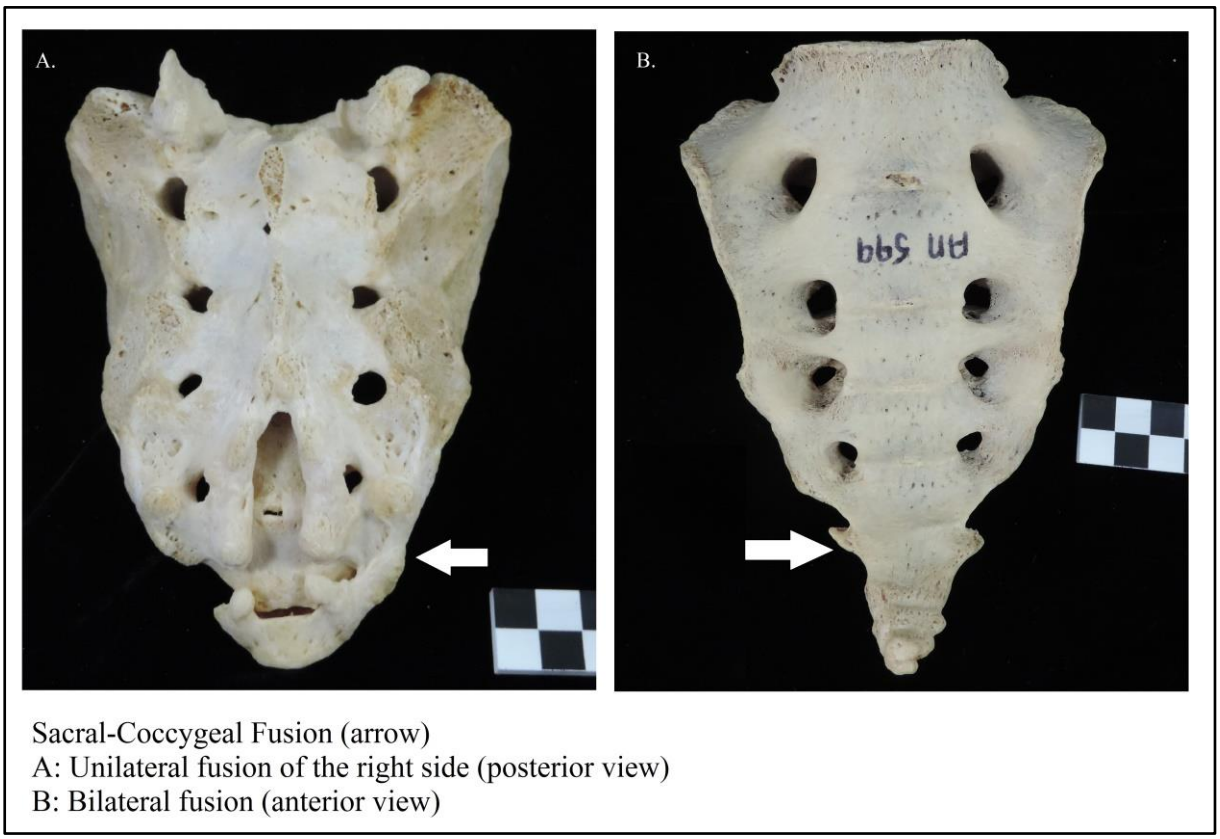
This study found that all of the specimens in the selection had TLTV ($f = 35$) at the thoracolumbar junction and at least one additional random congenital defect in the vertebral column. Based on the finding, it can be claimed that an association exists between TLTV and other congenital defects of the vertebral column. It is deduced that when TLTV is present in the vertebral column of an individual, it will be associated with a defect in the vertebral column.

Despite the association between TLTV and other defects in the vertebral column, there is not enough information to predict the specific type of defect that will develop in the vertebral column. It can only be claimed that the individual will be affected by at least one other defect in the vertebral column. The exact cause of the association remains uncertain.

4.2.SACRO-COCCYGEAL FUSION

Sacro-coccygeal fusion (Figure 4.2-1) is characterised by ankylosis of the coccyx and the sacrum. Fusion of the coccyx to the sacrum was the least observed ($f = 5$) congenital defect in the selection, featuring in 14.29% of the selection (Table 3.1-1).

This study observed that all the cases of sacro-coccygeal fusion in this study were associated with TLTV (Figure 3.3-1). Transitional vertebrae result from overlapping developmental fields, whereas sacro-coccygeal fusion results from a unilateral or bilateral shift of the segments. As both defects result from border shifts of the developmental segments, it is likely that the same shift will result in both defects if the shift occurs through the vertebral column. Sacro-coccygeal fusion results from the cranial shift during development. From the observations it can be claimed that sacro-coccygeal fusion is a defect that results from the cranial shift of vertebral segments. The shift is associated with segmental shifts at the thoracolumbar junction that present as transitional vertebrae at the thoracolumbar junction.



Sacral-Coccygeal Fusion (arrow)
A: Unilateral fusion of the right side (posterior view)
B: Bilateral fusion (anterior view)

Figure 4.2-1: Sacro-coccygeal fusion

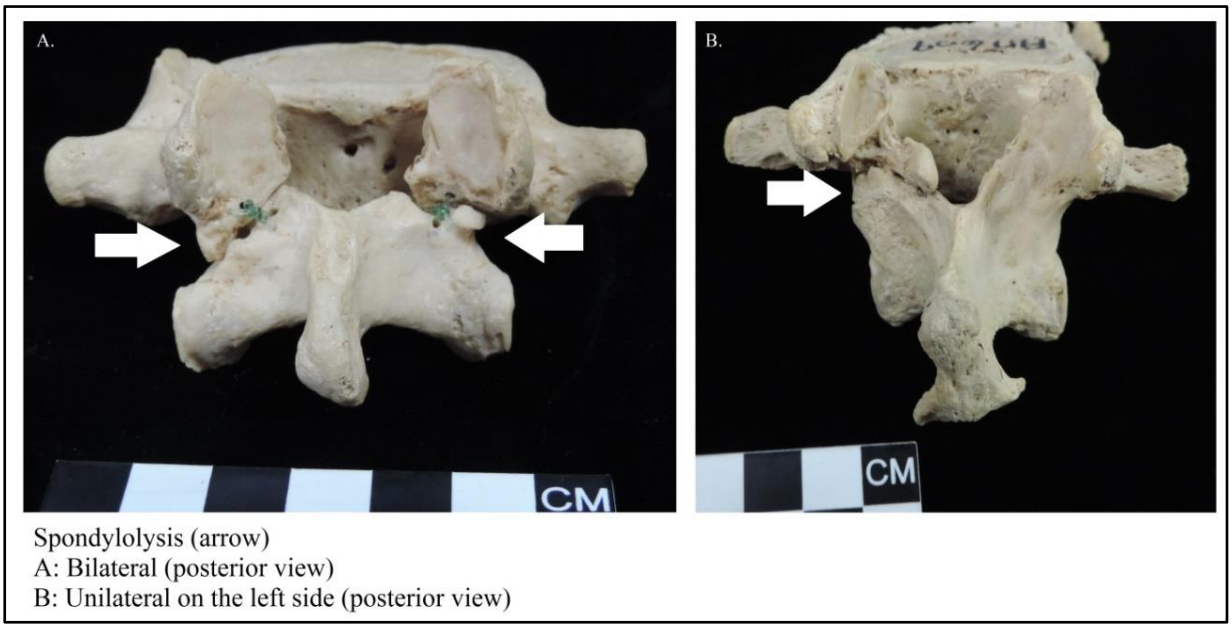
In the subset of specimens from the selection with sacro-coccygeal fusion, spina bifida was present with sacro-coccygeal fusion in one specimen (Figure 1.3-3). The features showed a unilateral segmental shift on the right side resulting in the ankylosis of the coccyx to the sacrum. Despite the overlapping features of sacro-coccygeal fusion and spina bifida in one case study, there is not enough evidence to support that a direct association between sacro-coccygeal fusion and spina bifida is present. Instead, an indirect association between sacro-coccygeal fusion and spina bifida is observed as both defects are associated with TLTV in all

observed cases. It is more likely that the overlapping defects of spondylolysis and spina bifida resulted by chance.

Subsequent observations showed that type IIa lumbarisation was associated with sacro-coccygeal fusion in a different case ($f = 1$) from the selection. The association between the defects likely resulted from the same cranial border shift that expanded to both the lumbosacral and sacro-coccygeal region. Both sacro-coccygeal fusion and lumbarisation require a caudal shift of the developing somite segments. The scarcity of specimens that relate this phenomenon can be explained by the relatively few specimens in the selection identified with lumbarisation ($f = 2$) and sacro-coccygeal fusion ($f = 5$). From this perspective it could be argued that one of two lumbarisation cases observed showed association with sacro-coccygeal fusion, although more information regarding case studies is required.

4.3.SPONDYLOLYSIS

Spondylolysis (Figure 4.3-1) is a defect in vertebrae characterised by the separation of the pars interarticularis in the neural arch. Spondylolysis was observed in eight specimens ($f = 8$) from the selection (Table 3.1-1).



Spondylolysis (arrow)
A: Bilateral (posterior view)
B: Unilateral on the left side (posterior view)

Figure 4.3-1: Spondylolysis

In the subset of specimens with spondylolysis, TLTV were observed in all cases (Figure 3.3-1). TLTV are caused by overlapping developmental fields of the last thoracic and first

lumbar segments. Based on the observation, it is claimed that spondylolysis is associated with border shift at the thoracolumbar junction that result in TLTV.

Unilateral spondylolysis on the right side of L5 was associated with spina bifida (S3-S5) in the sacrum in one specimen from the selection (Figure 3.3-1). Both spondylolysis and spina bifida are defects that are caused by the incomplete formation of the neural tube. Subsequent observations of a different specimen show that type IIa sacralisation was associated with unilateral spondylolysis on the left side in one specimen from the selection. The mechanism of the associations observed in the two cases remains unknown. Not enough corresponding cases were observed to claim that a direct association between sacralisation and spondylolysis or spina bifida and spondylolysis exists. Statistically it is more likely to suggest that the overlap between the defects in the cases is related to chance. An indirect association is, however, present between sacralisation and spondylolysis or spina bifida and spondylolysis. This claim is based on the observation that all the specimens with sacralisation, spina bifida and spondylolysis have TLTV.

4.4.NTD'S

NTD's in the vertebral column are anomalies of the neural arch that result from disrupted closure of the posterior neural pore (Figure 4.4-1). In vertebrae, it can be morphologically described that the spinous process of a vertebra is absent or the bilateral segments of the spinous process did not properly fuse during ossification. NTD's are the second most frequent congenital defect in the vertebral column observed in specimens from the selection ($f = 16$)(Table 3.1-1). It may occur that an individual has more than one type of NTD in the vertebral column.

Defective closure of the posterior neural arch was often observed at the distal points of the vertebral column, specifically C1 (Figure 4.1-1: B) and the lower sacral vertebrae (Figure 4.4-1: A). In all cases of NTD's observed, the defect was located at a regional junction in the vertebral column.

All the cases in the subset of specimens with at least one random NTD in the vertebral column had TLTV. It can, therefore, be claimed that random NTD's in the vertebral column are associated with cranial-caudal border shifts at the thoracolumbar junction that result in TLTV. The mechanism of the association remains uncertain.

Evaluation was conducted to evaluate association between random NTD's in the vertebral column and other defects considered in the study. As has been discussed in prior sections (4.2 & 4.3), spina bifida is the only NTD observed in this study to be associated with spondylolysis in one case and sacro-coccygeal fusion in another case.

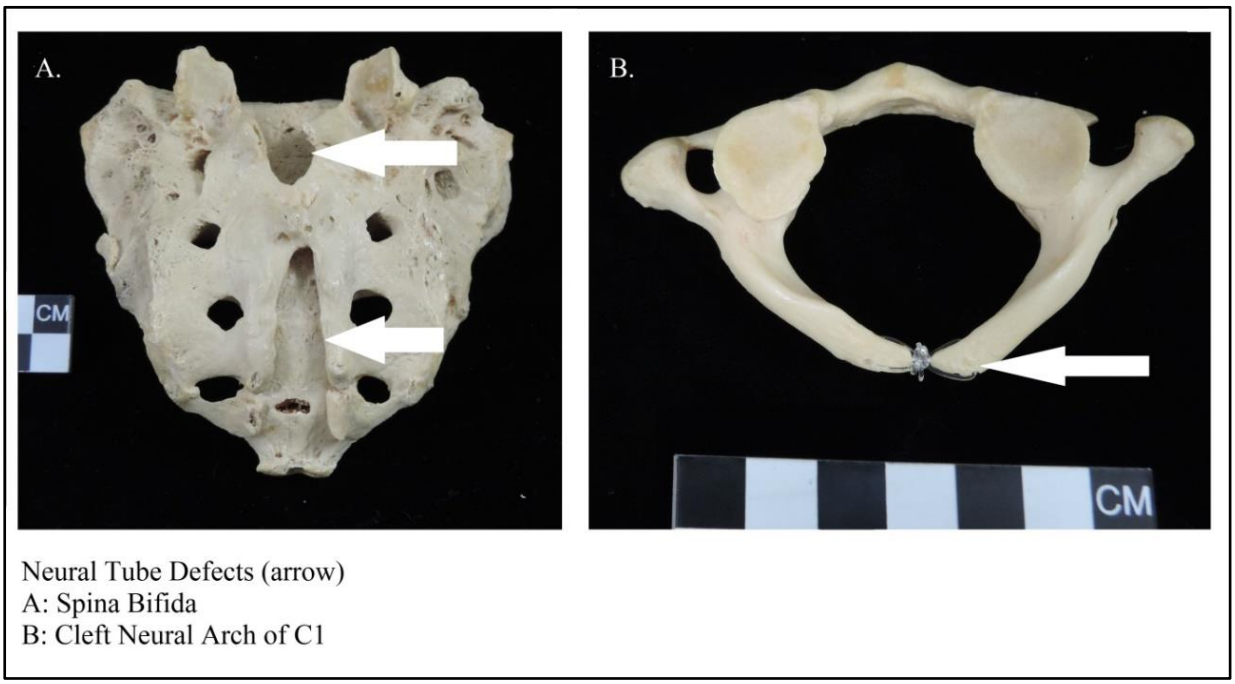


Figure 4.4-1: NTD's (A- Spina bifida in sacrum, B- Cleft in posterior neural arch of C1)

Subsequent observations show that random NTD's in the vertebral column were associated with LSTV in five cases in the selection. In this study, NTD's ($f = 16$) and LSTV ($f = 14$) were the most frequently observed defects located in the vertebral column. It is statistically possible that a subset of the specimens for both groups were bound to overlap. The relatively higher overlap of cases is likely due to the relatively higher group sizes of NTD's and LSTV compared to spondylolysis and sacro-coccygeal fusion. The direct mechanism between the associations remains unknown. An indirect association between NTD's and LSTV is, however, present as all the cases of specimens with NTD's and LSTV have transitional vertebrae at the thoracolumbar junction. The specified type of NTD observed was not significant.

4.5.LSTV

Transitional vertebrae at the lumbosacral junction are referred to as LSTV. Transitional vertebrae result from overlapping developmental field that are caused by the cranial or caudal shift of somites. The shift can be unilateral or bilateral.

It was observed that all specimens with LSTV present had TLTV. Based on the observation, it is claimed that an association is present between LSTV and TLTV. This association is very likely as both defects result from border shifting during development. If the shift that takes place at the thoracolumbar junction forming the TLTV expands to the lumbosacral junction, LSTV will develop. The association between LSTV and other defects in the vertebral column evaluated in this study has already been discussed in prior sections.

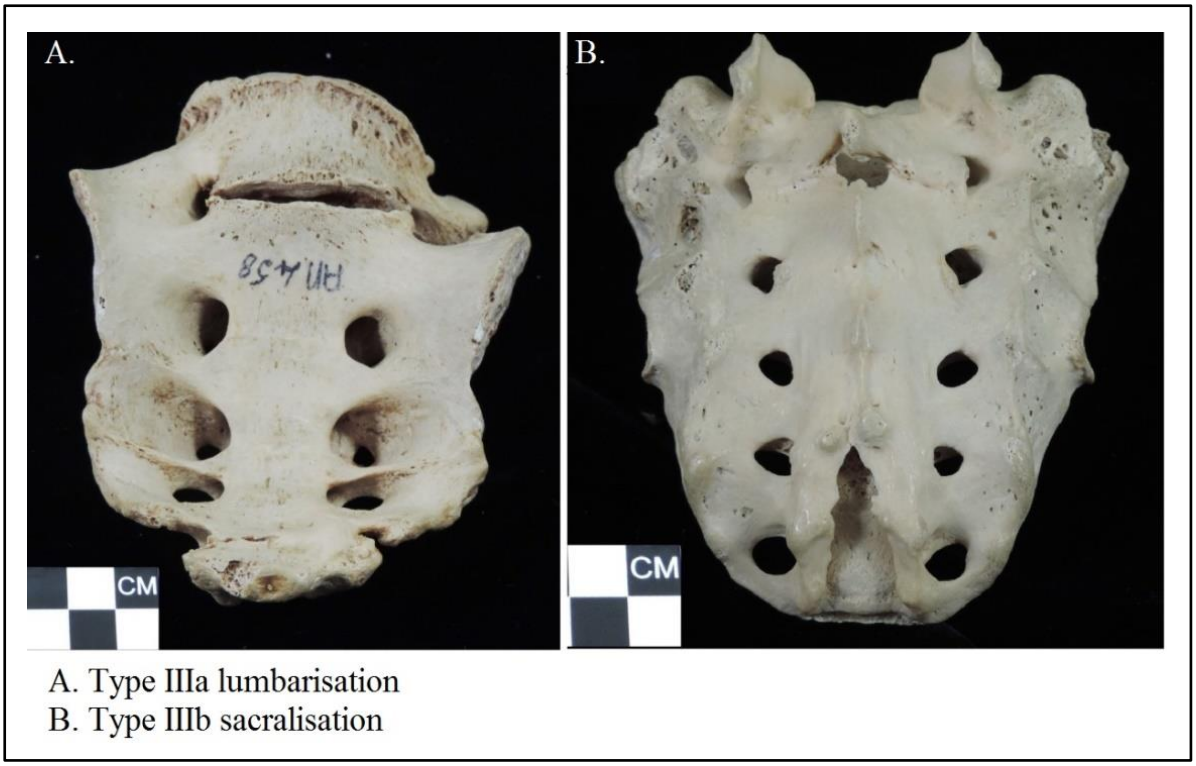


Figure 4.5-1: LSTV

It was noted during evaluation that not all cases of TLTV necessarily resulted in LSTV, although in these cases other defects were present in the vertebral column.

CONCLUSION

Unlike the other congenital defects evaluated in this study, very little is known about transitional vertebrae at the thoracolumbar junction. Transitional vertebrae at the thoracolumbar junction can be identified by the overlapping thoracic and lumbar features present in the vertebrae. There are many variations that may result from the overlapping developing fields that result in TLTV, both unilateral and bilateral. In this study, TLTV were classified according to the functional region that it is located in. This study concludes that TLTV are associated with other congenital defects in the vertebral column, specifically: LSTV, NTD's, spondylolysis and sacralisation of the coccyx. The association between TLTV and other congenital defects provides an indirect association between defects simultaneously present in the vertebral column.

REFERENCE LIST

Ahmad, F.U. & Mahapatra, A., 2009. Neural tube defects at separate sites: further evidence in support of multi-site closure of the neural tube in humans. *Surgical Neurology*, 71(3), pp.353–356.

Alton, T., Patel, A., Lee, M. & Chapman, J., 2014. Pediatric cervical spondylolysis and American football. *Spine Journal*, 14(6), pp.1–5. Available at: http://dx.doi.org/10.1016/j.spinee.2013.09.037.

Asher, R., Lin, K., Kardjilov, N. & Hautier, L., 2011. Variability and constraint in the mammalian vertebral column. *Journal of Evolutionary Biology*, 24(5), pp.1080–1090.

Attiah, M., Macyszyn, L. & Cahill, P., 2014. Management of spondylolysis and spondylolisthesis in the pediatric population: A review. *Seminars in Spine Surgery*, 26(4), pp.230–237. Available at: http://dx.doi.org/10.1053/j.semss.2014.09.005.

Barnes, E., 2012. *Atlas of Developmental Field Anomalies of the Human Skeleton*, New Jersey: John Wiley & Sons Inc.

Bauer, K.A., George, T.M., Enterline, D.S., Stottmann, R.W., Melvin, E.C., Siegel, D., Samal, S., Hauser, M.A., Klingensmith, J., Nye, J.S., et al., 2002. A Novel Mutation in the Gene Encoding Noggin is Not Causative in Human Neural Tube Defects. *Journal of Neurogenetics*, 16(1), pp.65–71. Available at: http://informahealthcare.com/doi/abs/10.1080/01677060290024628.

Botto, L., Moore, C., Khoury, M. & Erickson, J., 1999. Neural Tube Defects. *The New England Journal of Medicine*, 341(20), pp.1509–1519.

Byrd, S.E. & Comiskey, E.M., 2016. Postnatal Maturation and Radiology of the Growing Spine. *Neurosurgery Clinics of North America*, 18(2007), pp.431–461.

Carrino, J.A., Campbell, P.D.J., Lin, D.C., Morrison, W.B., Schweitzer, M.E., Flanders, A.E., Eng, J. & Vaccaro, A.R., 2011. Effect of spinal segment variants on numbering vertebral levels at lumbar MR imaging. *Radiology*, 259(1), pp.196–202.

Chang, H.L., Park, C.M., Kyeong, A.K., Suk, J.H., Hae, Y.S., Baek, H.K. & Jung, H.K., 2007. Identification and prediction of transitional vertebrae on imaging studies: Anatomical significance of paraspinal structures. *Clinical Anatomy*, 20(8), pp.905–914.

Colloca, C., Gunzburg, R., Freeman, B., Szpalski, M., Afifi, M. & Moore, R., 2012. Biomechancial quantification of pathologic manipulable spinal lesions: An in vivo ovine model of spondylolysis and intervertebral disc degeneration. *Journal of Manipulative and Physiological Therapeutics*, 35(5), pp.354–366. Available at: http://dx.doi.org /10.1016/j.jmpt.2012.04.018.

Copp, A., Stanier, P. & Greene, N., 2013. Neural tube defects: Recent advances, unsolved questions, and controversies. *The Lancet Neurology*, 12(8), pp.799–810. Available at: http://dx.doi.org/10.1016/S1474-4422(13)70110-8.

Dias, M.S., 2007. Normal and Abnormal Development of the Spine. *Neurosurgery Clinics of North America*, 18(3), pp.415–429.

Drake, R., Vogl, W. & Mitchell, A., 2009. *Gray's Anatomy for Students* 2nd ed., Churchill Livingstone.

Forseen, S.E., Gilbert, B.C., Patel, S., Ramirez, J. & Borden, N.M., 2015. Use of the Thoracolumbar Facet Transition as a Method of Identifying the T12 Segment. *Spine*, 4(2), pp.10–13.

Freund, J.E., 2011. *Modern Elementary Statistic* 12th ed., London: Pearson Prentice Hall.

Greene, N.D.E. & Copp, A.J., 2009. Development of the vertebrate central nervous system: Formation of the neural tube. *Prenatal Diagnosis*, 29(4), pp.303–311.

Greene, N.D.E., Stanier, P. & Copp, A.J., 2009. Genetics of human neural tube defects. *Human Molecular Genetics*, 18(2), pp.113–129.

Guerriero, Q.F., Arnell, R.E. & Irwin, J.B., 1940. Pelicephalography: an analysis of 503 selected cases. *Southern Medical Journal*, (33), pp.840–844.

Hansen, J.T., 2010. *Netter's Clinical Anatomy* 2nd ed. E. O'Grady, ed., Philidelphia: Saunders Elsevier.

Kershenovich, A., Macias, O., Syed, F., Davenport, C., Moore, G. & Lock, J.H., 2015. Conus medullaris level in vertebral columns with lumbosacral transitional vertebra. *Neurosurgery*, 78(1), pp.62–70.

Khairnar, K.B. & Rajale, M.B., 2013. Sacralisation of Lumbar Vertebrae. *Inidan Journal of Basic & Applied Medical Research*, 2(6), pp.510–514. Available at: www.ijbamr.com.

Kim, H.K. & Laor, T., 2010. Bilateral congenital cervical spondylolysis. *Pediatric Radiology*, 40(1), p.132.

Konin, G.P. & Walz, D., 2010. Lumbosacral transitional vertebrae: Classification, imaging findings, and clinical relevance. *American Journal of Neuroradiology*, 31(10), pp.1778–1786.

Kothari, C., 2004. *Research methodology: methods and techniques* 3rd ed., New Delhi: New Age International Ltd. Publishers. Available at: http://196.29.172.66:8080/jspui/bitstream/123456789/2574.

Lee, G.W., Ryu, J.H., Kim, J. Do, Ahn, M.W., Kim, H.J. & Yeom, J., 2015. Comparison of lumbar discectomy alone and lumbar discectomy with direct repair of pars defect for patients with disc herniation and spondylolysis at the nearby lumbar segment. *Spine Journal*, 15(10), pp.2172–2181. Available at: http://dx.doi.org/10.1016/j.spinee.2015.06.006.

Lurie, I., 2016. Distal trisomy 3q as a risk factor for neural tube defects. *Taiwanese Journal of Obstetrics and Gynecology*, 55(5), pp.769–770. Available at: http://linkinghub.elsevier.com/retrieve/pii/S102845591630119X.

Mahato, N.K., 2010. Morphological traits in sacra associated with complete and partial lumbarization of first sacral segment. *Spine Journal*, 10(10), pp.910–915.

Marawar, S., 2014. The radiographic parameters for the prediction of spondylolysis and spondylolisthesis. *Seminars in Spine Surgery*, 26(4), pp.219–224. Available at: http://dx.doi.org/10.1053/j.semss.2014.09.003.

Martínez-Frías, M.L., Urioste, M., Bermejo, E., Sanchís, A. & Rodríguez-Pinilla, E., 1996. Epidemiological analysis of multi-site closure failure of neural tube in humans. *American Journal of Medical Genetics*, 66(1), pp.64–68.

Masnicová, S. & Beňuš, R., 2003. Developmental anomalies in skeletal remains from the Great Moravia and Middle Ages cemeteries at Devín (Slovakia). *International Journal of Osteoarchaeology*, 13(5), pp.266–274.

McAnany, S., Cho, S., Qureshi, S. & Hecht, A., 2014. A commentary on cervical spondylolysis in the contact athlete. *Spine Journal*, 14(6), pp.e7–e8. Available at: http://dx.doi.org/10.1016/j.spinee.2014.02.026.

McMahon, J., Takada, S., Zimmerman, L., Fan, C.M., Harland, R. & McMahon, A., 1998. Noggin-mediated antagonism of BMP signaling is required for growth and patterning of the neural tube and somite. *Genes and Development*, 12(10), pp.1438–1452.

Menga, E., Jain, A., Kebaish, K., Zimmerman, S. & Sponseller, P., 2013. Anatomic Parameters for Intralaminar Screw Repair of Spondylolysis. *The Spine Journal*, 13(9), pp.75–76. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1529943013009790.

Merbs, C.F., 1974. The effects of cranial and caudal shift in the vertebral columns of northern populations. *Arctic Anthropology*, (11), pp.12–19.

Metkar, U., Shepard, N., Cho, W. & Sharan, A., 2014. Conservative management of spondylolysis and spondylolisthesis. *Seminars in Spine Surgery*, 26(4), pp.225–229. Available at: http://dx.doi.org/10.1053/j.semss.2014.09.004.

Mobasheri, E., Keshtkar, A. & Golalipour, M., 2010. Maternal folate and vitamin b(12) status and neural tube defects in northern iran: a case control study. *Iranian journal of pediatrics*, 20(2), pp.167–73. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3446032&tool=pmcentrez&rendertype=abstract.

Moore, K., Agur, A. & Dalley, A., 2010. *Essential Clinical Anatomy* Fourth., USA: Lippincott Williams & Wilkins.

Myrianthopoulos, N.C. & Melnick, M., 1987. Studies in neural tube defects. I. Epidemiologic and etiologic aspects. *American journal of medical genetics*, 26(4), pp.783–96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3296753.

Nakajima, A., Usui, A., Hosokai, Y., Kawasumi, Y., Abiko, K., Funayama, M. & Saito, H., 2014. The prevalence of morphological changes in the thoracolumbar spine on whole-spine computed tomographic images. *Insights into Imaging*, 5(1), pp.77–83.

Netter, F., 2011. *Atlas of Human Anatomy* 5th ed. J. T. Hansen, B. Benniger, J. K. Brueckner, S. W. Carmichael, N. A. Granger, & R. S. Tubbs, eds., Philadelphia: Elsevier Saunders.

Oishi, A., Sodeyama, T. & Yanagisawa, S., 2016. *Therapy Treatment for Juvinile Spondylolysis*, The Chartered Society of Physiotherapy.

Oostra, R.J., Hennekam, R.C.M., De Rooij, L. & Moorman, A.F.M., 2005. Malformations of the axial skeleton in Museum Vrolik I: Homeotic transformations and numerical anomalies. *American Journal of Medical Genetics*, 134 A(3), pp.268–281.

Overley, S.C., McAnany, S.J., Andelman, S., Kim, J., Cho, S.K., Qureshi, S.A. & Hecht, A., 2016. Return to Play in Adolescent Athletes with Symptomatic Spondylolysis without Listhesis: a Meta-Analysis. *The Spine Journal*, 16(10), pp.S376–S377. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1529943016308142.

Page, R.R. & Page, B.J., 2013. *Requirements and Guidelines for Report Writing*, Tygerberg: Stellenbosch University.

Paraskevas, G., Tzika, M. & Kitsoulis, P., 2013. Lumbosacral Transitional Vertebra Associated with sacral spina bifida occulata: A Case Report. *ACTA Medica International Journal*, 56(3), pp.126–129.

Peer, K.S. & Fascione, J.M., 2007. Spondylolysis A Review and Treatment Approach. *Orthopaedic Nursing*, 26(2), p.104. Available at: http://journals.lww.com/orthopaedicnursing/Abstract/2007/03000/Spondylolysis__A_Review_and_Treatment_Approach.7.

Puvirajesinghe, T.M. & Borg, J.-P., 2015. Neural tube defects: from a proteomic standpoint. *Metabolites*, 5(1), pp.164–83. Available at: www.mdpi.com/journal/metabolites/.

Racusin, D., Villarreal, S., Antony, K., Harris, R., Mastorbattistia, J., Shamshiraz, A., Belfort, M. & Aagaard, K., 2015. Detection of open neural tube defects (ONTD) as an opportunity for intervention. *American Journal of Obstetrics and Gynecology*, 212(1), pp.S312–S313. Available at: http://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=00029378&id=doi:10.1016/j.ajog.2014.10.840&atitle=Detection+of+open+neural.

Rawls, A. & Fisher, R., 2010. Development and functional anatomy of the spine. In *The Genetics and Development of Scoliosis*. pp. 21–46.

Rosa, R.F.M., Trevisan, P., Rosa, R.C.M., Lorenzen, M.B., Zen, P.R.G., Oliveira, C.A., Graziadio, C. & Paskulin, G.A., 2013. Trisomy 18 and neural tube defects. *Pediatric Neurology*, 49(3), pp.203–204. Available at: http://dx.doi.org/10.1016/j.pediatrneurol.

Rustagi, T., Lavelle, W. & Tallarico, R., 2014. Surgical management of adult spondylolysis and spondylolisthesis. *Seminars in Spine Surgery*, 26(4), pp.238–246. Available at: http://dx.doi.org/10.1053/j.semss.2014.09.006.

Samreen, P., Shashikala, L. & Rohini, K., 2012. LUMBARIZATION OF FIRST SACRAL VERTEBRA: A CASE REPORT. *International Journal of Basic and Applied Medical Sciences*, 2(2), pp.154–157.

Savage, C., 2005. *Lumbosacral transitional vertebrae: classification of variation and association with low back pain*. Univeristy of Missouri. Available at: https://mospace.umsystem.edu/xmlui/handle/10355/4318.

Sekharappa, V., Amritanand, R., Krishnan, V. & David, K.S., 2014. Lumbosacral transition vertebra: Prevalence and its significance. *Asian Spine Journal*, 8(1), pp.51–58.

Shaefer, A., 2015. Folate vs. Folic Acid. *Healthline*. Available at: www.healthline.com /health/food-nutrition/folate [Accessed November 11, 2016].

Shaw, G.M., Carmichael, S., Yang, W., Finnell, R., Midttun, Ø. & Ueland, P.M., 2009. Mid-Pregnancy Cotinine and Risks of Orofacial Clefts and Neural Tube Defects. *Journal of Pediatrics*, 154(1), pp.17–19.

Tague, R., 2011a. Fusion of Coccyx to Sacrum in Humans : Prevalence, Correlates, and Effect on Pelvic Size, With Obstetrical and Evolutionary Implications. *American Journal of Physical Anthropology*, 145(January), pp.426–437.

Tague, R., 2011b. Sacralization is not associated with elongated cervical costal process and cervical rib. *Clinical Anatomy*, 24(2), pp.209–217.

Taylor, T., Farkouh, R., Graham, J., Colligs, A., Lindemann, M., Lynen, R. & Candrilli, S.D., 2011. Potential reduction in neural tube defects associated with use of Metafolin-fortified oral contraceptives in the United States. *American Journal of Obstetrics and Gynecology*, 205(5), p.460.e1-460.e8. Available at: http://dx.doi.org/10.1016/j. ajog.2011.06.048.

Tekkök, I., 2005. Triple neural tube defect - Cranium bifidum with rostral and caudal spina bifida - Live evidence of multi-site closure of the neural tube in humans. *Child's Nervous System*, 21(4), pp.331–335.

Thawait, G.K., Chhabra, A. & Carrino, J.A., 2012. Spine Segmentation and Enumeration and Normal Variants. *Radiologic Clinics of North America*, 50(4), pp.587–598. Available at: http://dx.doi.org/10.1016/j.rcl.2012.04.003.

Uçar, B.Y., Uçar, D.E., Bulut, M., Azboy, I. & Demirtaş, A., 2013. Lumbosacral Transitional Vertebrae in Low Back Pain Population. *Spine*, 2(1), pp.2–4. Available at: http://dx.doi.org/10.4172/2165-7939.1000125.

Wilson, D., 2015. Pre-conception Folic Acid and Multivitamin Supplementation for the Primary and Secondary Prevention of Neural Tube Defects and Other Folic Acid-Sensitive Congenital Anomalies. *Journal of Obstetrics & Gynaecology*, 37(324), pp.534–549.

Wilson, D., 2014. Prenatal screening, diagnosis, and pregnancy management of fetal neural tube defects. *Journal of Obstetrics & Gynaecology*, 36(10), pp.1117–1130.

Wright, J., Balaji, V. & Montgomery, A.S., 2013. (ii) Spondylolysis & spondylolisthesis. *Orthopaedics and Trauma*, 27(4), pp.195–200. Available at: http://dx.doi.org/10.1016/j.mporth.2013.07.002.