International Journal of Research in Medical Sciences
Nazir S et al. Int J Res Med Sci. 2014 Feb;2(1):293-299
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: 10.5455/2320-6012.ijrms20140256

Research Article

Histological development of human foetal shoulder joint

Sabia Nazir*, Sunanda Raina, Zahid Bashir, Shamima Bano, Shaheen Shadad, Javed A. Khan, Yasmeen Jan

Department of Anatomy, Government Medical College, Srinagar, J & K, India

Received: 5 December 2013
Revised: 21 January 2014
Accepted: 25 January 2014

***Correspondence:**
Dr. Sabia Nazir,
E-mail: dr.sabianazir@gmail.com

ABSTRACT

Background: Shoulder joint is a multiaxial, diarthrodial joint of ball and socket variety. The various components of shoulder joint i.e. capsule, various ligaments, joint cavity and synovial tissue start developing in early embryonic and foetal life roughly in between 6th to 12th week of foetal development. The present study aims to analyze the sequences of development of various structures of shoulder joint in human embryo and compare the findings with other observers to gain some insight regarding its development and an attempt is made to correlate these observations clinically to analyze cause and management of recurrent shoulder dislocations.
Methods: Shoulder joints of 32 foetuses collected from areas in and around Jammu were dissected properly and decalcified in Gooding and Stewart's solution. Sections were cut after obtaining blocks by paraffin wax embedding method. Slides were stained using Haematoxylin and Eosin, Masson's trichrome and orcein staining and important findings were documented.
Results: The bony structures - head of humerus and glenoid fossa and joint cavity appear by 10 weeks which prolongs into bicipital sulcus by 12-1/2 weeks. Synovial tissue appears by 10 weeks and synovial villi appear by 14 weeks. Glenoid labrum, capsular ligament, coracohumeral ligament and superior glenohumeral ligament are seen by 10 weeks. Middle glenohumeral ligament is seen at 12-1/2 weeks while inferior glenohumeral ligament is seen at 14 weeks. Tendon of biceps is seen at 10 weeks.
Conclusions: By 10 weeks of gestational age various structures of shoulder joint develop in situ, resembling in form and arrangement as those of adults. From these early stages, development proceeds rapidly to achieve adult characteristics. There are no intermediate stages in between where structures similar to those of lower forms i.e. syn/amphi artroses appear temporarily.

Keywords: Shoulder joint, Capsular ligament, Synovial tissue

INTRODUCTION

Shoulder joint is formed by articulation of head of humerus with glenoid fossa of scapula. Humeral head is about 4 times the size of glenoid fossa.[1] By 6 weeks, first hyaline cartilage models foreshadowing the bones of shoulder joint are formed by chondrocytes.[2] When skeletal elements are formed, there are areas in between which do not undergo any change into cartilage or bone. These areas persist as joint interzones and are sites for development of future joints. Each interzone passes through a three layered stage with two chondrogenous layers and an intermediate loose layer. The growth of cartilagenous elements compresses the central part of interzone and the cavity appears in its circumferential portion. The cavity expands gradually and extends towards centre of joint. The cells in area of cavitation undergo liquefaction. Macrophages present in periphery of joint interzones produce lytic enzymes which could be involved in cavitation process.[3]

The synovial mesenchyme gives rise to synovial and subsynovial tissue and all intracapsular structures including ligaments, tendons and fibrocartilages. The synovial folds are part of synovial mesenchyme dissected out by secondary extensions of cavities as they spread over cartilages.[4] Mesenchymal cells lining the articular surfaces and capsule of shoulder joint are flattened and form synovial membrane by 8 to 10 weeks.[2] Synovial villi develop by 11 weeks.[2]

Mesenchymal tissue surrounding the developing joint which is continuous with perichondrium forms a sleeve like membrane which eventually transforms into capsular ligament by 9 weeks.[5] It develops four local thickenings to form coracohumeral ligament and three glenohumeral ligaments. Coracohumeral ligament develops by 6 1/2weeks[6] to 15 weeks.[7] Glenohumeral ligaments develop from 12 weeks[2] to 16 weeks.[8] These ligaments limit lateral rotation at shoulder joint and act as check reins.[9] The joint is strengthened by group of short muscles which blend with capsule and form rotator cuff. These have been described as true active ligaments by Poirier and Charpy as cited by Sarrafian.[10] Glenoid labrum is fibrocartilagenous rim surrounding glenoid fossa. It deepens the cavity and contributes to stability of joint.[11] It becomes fibrocellular by 12 weeks and fibrocartilagenous at 16 weeks.[8]

So many facts are controversial about stage of development of shoulder joint. This work aims to provide adequate description of embryogenesis and histogenesis of the joint especially regarding capsule, joint cavity, various ligaments and synovial tissue.

METHODS

This study was conducted on 32 foetuses of various gestational ages obtained from 2005 to 2006 in Jammu (India) and areas surrounding it. The foetuses were obtained as products of abortions, still births and hysterotomy procedures. They were preserved in 10% formalin. The crown rump (CR) length of foetuses was measured with vernier calliper to determine their gestational age as per rule described by Hamilton, Boyd and Mossman.[12]

The crown rump lengths of foetuses studied along with their estimated gestational age are given in Table 1.

Table 1: The crown rump length of foetuses studied along with their estimated gestational age.

Sr. No.	Crown rump length (mm)	Calculated age (days)	Calculated age (wk-weeks, d-days)	Sex	No. of cases Studied
1.	53	72	10wk + 2d	Male	1
2.	58	75	10wk + 5d	Male	1
3.	75	86	12wk + 2d	Female	1
4.	77	88	12wk + 4d	Male	1
5.	95	100	14wk + 2d	Female	1
6.	98	102	14wk + 4d	Female	1
7.	110	110	15wk + 5d	Female	1
8.	112	111	15wk + 6d	Female	1
9.	115	113	16wk + 1d	Female	1
10.	117	114	16wk + 2d	Female	1
11.	118	115	16wk + 3d	Female	1
12.	120	116	16wk + 4d	Female	1
13.	128	122	17wk + 3d	1 Male, 1 Female	2
14.	132	124	17wk + 5d	Female	1
15.	133	125	17wk + 6d	Female	1
16.	134	126	18wk	Male	1
17.	135	126	18wk	1 Male, 1 Female	2
18.	136	127	18wk + 1d	Female	1
19.	140	130	18wk+4d	Female	1

20.	142	131	18wk+5d	Female	1
21.	143	132	18wk+6d	Female	1
22.	145	133	19wk	1 Male, 1 Female	2
23.	146	134	19wk+1d	Female	1
24.	168	148	21wk+1d	Male	1
25.	172	151	21wk+4d	Female	1
26.	195	166	23wk+5d	Male	1
27.	210	180	25wk+5d	Male	1
28.	220	183	26wk+1d	Female	1
29.	240	196	28wk	Female	1
Total specimen studied					32

Sex of foetus was determined and the pectoral girdle was separated from trunk. The area was dissected properly and dissected specimen was kept inside tissue capsule. Tissue capsule is a small container having holes in it, which allow fixative to enter inside when tissue is placed inside. This prevents loss of specimen during processing. Specimen was fixed by 10% formalin solution to impart firm consistency for period of 48 hours to 1 week - depending upon size of specimen. The specimen was decalcified by placing in Gooding and Stewart's 5% solution for 4-5 days. Bigger specimen was decalcified by treating with 5% nitric acid. The tissue was prepared for sectioning by using paraffin wax embedding method. After trimming paraffin wax block, sections of 7µ thickness were cut with rotary microtome. Both coronal and transverse sections were taken. Sections were transferred to water-bath to remove wrinkles. Sections were fixed on slides smeared with drop of Mayer's egg albumin. Then slides were stained with Haematoxylin and Eosin, Masson's trichrome and orcein. Slides were examined under light microscope and photographic documentation of important findings was done.

RESULTS

The foetuses are divided into six groups based on their stage of histological development as shown in Table 2.

Group I: 53 - 58 mm CR length (72-75 days or 10-11 weeks old)

The coronal section of foetus of 10-11 weeks reveals that skeletal elements are quite discernable. Humerus has slight constriction separating head from shaft. Upper end of humerus is less mature as compared to shaft. Scapula shows concave glenoid fossa and neck can be differentiated. Coracoid process is larger in size than acromion which is still cartilagenous. Joint cavity is clearly visualized. Tissue lining joint cavity is loose like synovial tissue and inferiorly it is reflected on neck of humerus laterally and medially it is attached to glenoid labrum. Capsule is seen as continuation of perichondrium and is made of collagen fibres. It is more cellular than fibrous (Figure 1). Shaft of humerus is ossifying which has reached distal to the attachment of latissmus dorsi and teres major muscle. Beyond ossification areas of hypertrophied cartilage can be seen. Periosteum of humerus has two layers – inner osteogenic and outer fibrous. A zone of increased density represents coracohumeral ligament (Figure 1). Acromioclavicular joint is seen. It is lined by flattened cells. Perichondrium from acromion extends to clavicle and serves the purpose of capsular ligament. Lateral end of clavicle is cartilagenous in nature.

Table 2: Foetuses divided into six groups depending on their stage of development.

Group No.	Crown rump length (mm)	Age (weeks)	No. of foetuses
I	53-58mm	10 to 11	2
II	75-98mm	12 to 14	4
III	110-128mm	15 to 17-1/2	8
IV	132-142mm	18 to 18-1/2	8
V	143-168mm	19 to 21	5
VI	172-240mm	22 to 28	5

Group II: 75 - 98 mm CR length (86-102 days or 12-14 weeks)

Frontal sections of foetus of 12 weeks reveal that ossification in humerus has extended further in shaft proximal to the attachment of latissmus dorsi and teres major muscle. Joint cavity is being surrounded by capsular ligament which is continuous with

perichondrium of humerus and scapula. Capsular ligament is thickened superiorly below the attachment of supraspinatus muscle between lesser tubercle and glenoid labrum depicting superior glenohumeral ligament (Figure 2). Inferiorly, between lesser tubercle and glenoid labrum middle glenohumeral ligament is seen as thickening in capsule. Frontal section of 14 weeks foetus show greater and lesser tubercles to be well distinguished. Ossification in shaft of humerus has extended up to level slightly distal to inferior synovial reflection. An important observation is presence of synovial villi in joint cavity (Figure 3). Biceps tendon contains more collagen fibres. It is densely collagenous in its centre. The cavity surrounding it communicates with joint cavity, hence, proving that it is an extension of joint cavity.

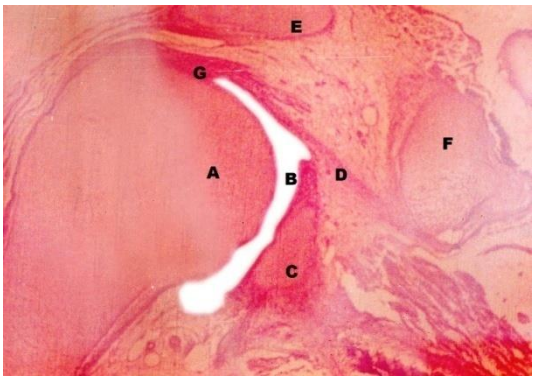

Figure 1: Coronal section of shoulder joint of foetus of 10 to 11 weeks. A) Head of humerus B) Joint cavity C) Glenoid fossa of scapula D) Coracohumeral ligament E) Acromion F) Coracoid process G) Capsular ligament - H & E x 20.

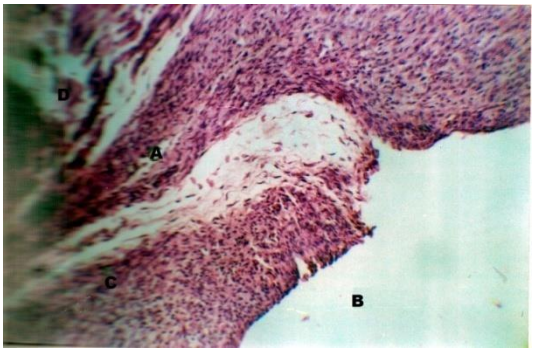

Figure 2: Superior glenohumeral ligament in foetus of 12 weeks. A) Superior glenohumeral ligament B) Joint cavity C) Capsule D) Supraspinatus muscle - H & E x 100.

Group III: 110 - 128 mm CR length (110-122 days or 15 to 17-1/2 weeks)

Frontal section of foetus of 15 weeks show increase in ossification of shaft of humerus. Periosteum becomes thicker due to increase in number of collagen fibres. In scapula, ossification has extended up to neck. Joint cavity has increased in size. Capsule has increased in thickness due to increase in number of collagen fibres. It is being strengthened by superior glenohumeral ligament. Transverse section of foetus of 16 weeks shows that ossification in scapula has extended up to base of acromion (Figure 4). Acromion and head of scapula are still cartilagenous. Glenoid labrum is vascularised at its margin and has become more fibrous. Transverse section of foetus of 17 weeks reveals that synovial membrane lining joint cavity has numerous synovial villi. Branching of synovial villi is seen and they are highly vascular.

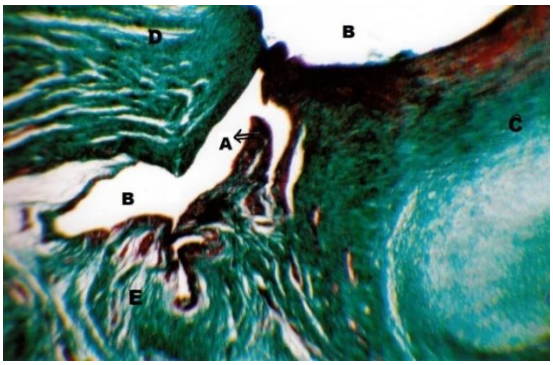

Figure 3: Synovial villi in joint cavity of foetus of 14 weeks. A) Synovial villi B) Joint cavity C) Glenoid fossa D) Capsular ligament E) Synovial tissue - Masson's trichrome x 50.

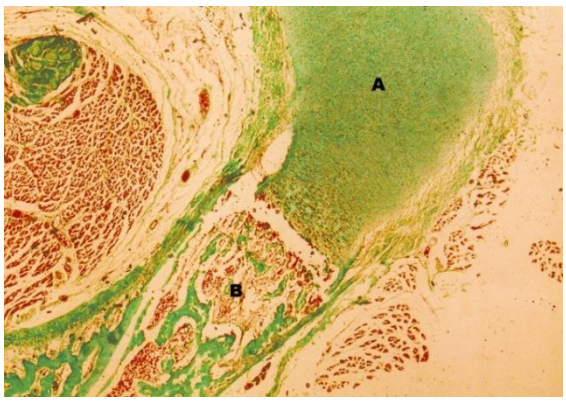

Figure 4: Extension of ossification in scapula up to base of acromion in foetus of 16 weeks. A) Acromion B) Spine of scapula - Masson's trichrome x 20.

Group IV: 132 - 142 mm CR length (124-131 days or 18 to 18-1/2 weeks)

Frontal sections of foetus of 18 weeks show that ossification within shaft has extended up to level of inferior synovial reflection. In scapula, ossification has reached beyond neck – almost up to glenoid fossa. Joint cavity is permeated by biceps tendon. So it is intracapsular and extrasynovial (Figure 5). Joint cavity has increased in size with synovial villi within it. Tendon of supraspinatus has become vascularised. Transverse section of foetus of 18-1/2 weeks show joint cavity has extended beyond glenoid labrum. Superomedially, it is attached beyond supraglenoid tubercle. Glenoid labrum is

vascularised throughout its extent including basal area. More fibrous tissue is present in glenoid labrum. It is fibrocellular rather than fibrocartilagenous.

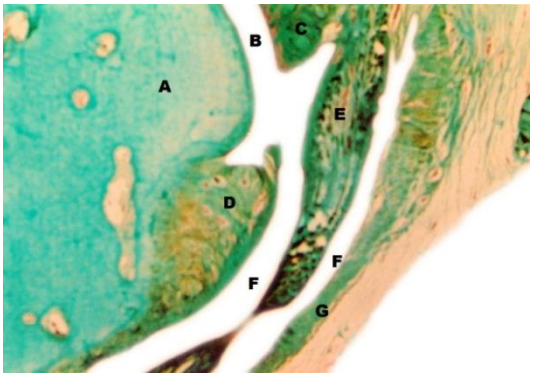

Figure 5: Biceps tendon permeating joint cavity in foetus of 18 weeks. A) Head of humerus B) Joint cavity C) Glenoid labrum D) Greater tubercle E) Tendon of biceps F) Cavity surrounding tendon of biceps G) Capsular ligament - Masson's trichrome x 20.

Group V: 143 - 168 mm CR length (132-148 days or 19-21 weeks)

Frontal section of foetus of 19 weeks reveals that ossification has extended in shaft of humerus slightly beyond the level of inferior synovial reflection. In scapula, ossification has reached the level of neck of scapula. Capsule has increased in thickness. Joint cavity has increased in size (Figure 6). It is lined by synovial membrane and synovial villi are seen inside. Shaft of humerus shows increase in thickness of bony collar.

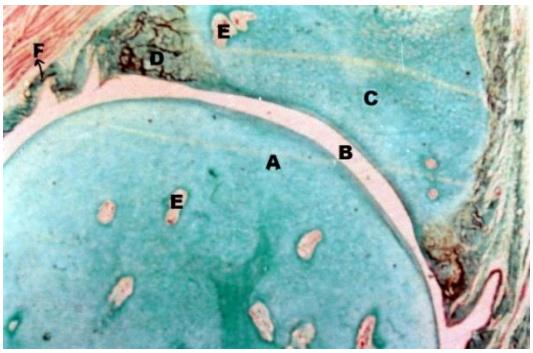

Figure 6: Joint cavity in foetus of 19 weeks. A) Head of humerus B) Joint cavity C) Glenoid fossa D) Glenoid labrum E) Cartilage canals F) Capsular ligament-Masson's trichrome x 20.

Group VI: 172 - 240 mm CR length (151 - 196 days or 22-28 weeks)

Frontal section of foetus of 22weeks shows synovial membrane lining joint cavity has increased in thickness. It has become more vascular. Ossification in shaft of humerus has extended beyond inferior synovial reflection. Bony collar around shaft of humerus has increased in thickness. Frontal section of foetus of 24-25 weeks shows that cartilage canals have reached articular surfaces of both head of humerus and glenoid fossa of scapula. Cartilage cells in deeper part of head of humerus are round while those near articular surface are spindle shaped. Elastic fibres could not be visualized in capsule, periosteum, biceps tendon or any other ligament.

DISCUSSION

In this work, skeletal elements like head of humerus with greater and lesser tubercles and intertubercular sulcus lodging biceps tendon are seen at about 10 weeks. These finding are in accordance with Laila M.[8] Same observations have been put forth by two studies[2,3] but at an earlier stage of 7 to 7-1/2 weeks. Coracoid process, acromion and spine of scapula are seen at 10 weeks. Glenoid fossa is deep and concave. This is in accordance with findings reported by Laila M.[8] in developing joints and Basmajian and Slonecker[13] in adult joints. By 12 weeks ossification in shaft of humerus extends up to level of insertion of latissmus dorsi and teres major muscle. In scapula ossification has extended up to neck. Same findings were put forth by Gardner and Gray.[2]

In present study, joint cavity is seen to be present by 10 weeks and it prolongs into bicipital sulcus by 12-1/2 weeks. Similar changes were noted in four studies.[2-4,8] The cavity continues to increase in size with subsequent stages of development. Once a cavity is formed, it remains approximately the same in relative size but gradually increases in absolute size. Hence cavitation is an active process.[3]

The synovial tissue makes its appearance by 10 weeks. It is difficult to differentiate it from capsular ligament in earlier stages. In synovial tissue the collagen fibres are irregularly arranged amongst cells. In capsule, collagen fibres run in parallel manner with rows of fibroblasts in-between. This is in accordance with Laila M.[8] Synovial tissue gets reflected on neck of humerus inferiorly by 10 weeks. It is more vascular near free margin of glenoid labrum and extends beyond glenoid lip to neck of scapula in upper part of joint. Synovial tissue is thicker, looser and more vascular in regions which do not lie below tendons. Similar findings were put forth by Gardner and Gray.[2] Synovial villi and folds are seen within joint cavity by 14 weeks. This finding corresponds with Andersen.[3] Villi are vascularised by 16 weeks. Collagenous core is seen within villus at 26 weeks. Many thick wavy closely packed collagen bundles form the core. These findings correspond with two studies.[4,8]

At 10 weeks glenoid labrum is well defined and is attached to margins of glenoid fossa. It is fibrocellular rather than fibrocartilagenous and contains collagen fibres which continue to increase with time. By 12-1/2 weeks capillaries can be seen in free margin of glenoid labrum. This is in accordance with Andersen.[3] No

fibrocartilage has been seen in glenoid labrum. Similar findings were put forth in three studies.[2,3,14] The superior part of glenoid labrum is associated with biceps tendon and appears as an extension of tendon. The results are in agreement with several studies.[2,3,8,15-17] No elastic fibres could be seen in glenoid labrum.[14]

Capsule becomes marked by 10 weeks. The capsule is more cellular than fibrous. Inferiorly, it is thick, richly cellular and more vascular. Similar findings have been noted by several studies.[2,3,5,8] No elastic fibres could be detected with certainty in capsule during foetal period with orcein. Coracohumeral ligament is seen by 10 weeks. Same has been noted in two studies.[3,6] Superior glenohumeral ligament is seen at 10 weeks. Middle glenohumeral ligament is seen as thickening in capsule by 12-1/2 weeks and inferior glenohumeral ligament is seen at 14 weeks. Ligaments are quite well defined but comprise largely of cells. Their collagen fibre content continues to increase with time. Transverse humeral ligament is seen between two tubercles and is continuous with capsular ligament. No fibrocartilage is seen within the ligament. Our findings correspond with Gardner and Gray.[2] No elastic fibres are visualized in any of these ligaments.

All muscles and tendons in vicinity of shoulder joint are well differentiated. Tendons contain strands of collagen. Biceps tendon is seen as rounded cord like structure which passes over humeral head to attach to superior labrum by 10 weeks. It is densely cellular. This is in accordance with three studies.[2,3,8] It increases in size due to deposition of collagen fibres. Blood vessels are noted within this tendon by 16 weeks whereas other tendons like those of supraspinatus and infraspinatus show vascularisation by 18 weeks. These findings are supported by three studies.[1,15,17] Acromioclavicular joint is seen by 10 weeks and is located much closer to acromion than to clavicle. Same has been noted by Gardner and Gray.[2] No fibrocartilage is seen in the joint at any stage of development.

CONCLUSION

It was found that the various components of shoulder joint are discernable by 10 weeks. Head of humerus with its two tubercles and intertubercular sulcus lodging the biceps tendon, acromion, coracoid process and spine of scapula are cartilagenous. Ossification occurs in shaft which with due course of time extends higher up. Periosteum of shaft of humerus has inner cellular and outer fibrous layers.

Joint cavity appears by 10 weeks. It is delineated by capsular ligament which is lined internally by synovial tissue. Synovial villi appear by 14 weeks and are vascularised by 16 weeks. They develop collagenous cores at 26 weeks and show branching. Joint cavity prolongs into bicipital sulcus by 12-1/2 weeks.

Glenoid labrum appears at 10 weeks and is fibrocellular rather than fibrocartilagenous. The biceps tendon extends proximally to get attached to supraglenoid tubercle and biceps tendon is continuous with labrum in its superior part. This explains association of labral detachment with recurrent shoulder dislocations in which all or part of superior labrum is avulsed from glenoid fossa along with origin of biceps tendon.

Capsule develops by 10 weeks and with increasing time the number of collagen fibres tends to increase. Coracohumeral and superior glenohumeral ligament appear by 10 weeks. Middle glenohumeral ligament develops by 12-1/2 weeks. Inferior glenohumeral ligament develops by 14 weeks. Glenoid labrum, biceps tendon and glenohumeral ligaments form a complete ring and this ring can be considered to be prime factor in stability of shoulder joint.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Hulstyn MJ, Fadale PD. Arthroscopic anatomy of the shoulder. Orthopedic Clinics of North America. 1995;26(4):597-612.
2. Gardner E, Gray DJ. Prenatal development of the human shoulder and acromioclavicular joints. American Journal of Anatomy. 1953;92:219-76.
3. Andersen H. Histochemistry and development of the human shoulder and acromioclavicular joints with particular reference to the early development of the clavicle. Acta Anatomy. 1963;55:124-65.
4. Haines RW. The development of joints. Journal of Anatomy. 1947;81:33-55.
5. Neale RM. The alleged transmigration of the long tendon of biceps brachii The Anatomical Records. 1937;67:205-18.
6. Lewis WH. The development of arm in man. American Journal of Anatomy. 1901;1:145-84.
7. Fealy S, Rodeo SA, Dicarlo EF, O'Brien SJ. The developmental anatomy of the neonatal glenohumeral joint. Journal of Shoulder and Elbow Surgery. 2000;9:217-22.
8. Laila M, Aboul Mahasen, Sohair A. Sadek Developmental, Morphological and Histological studies on structures of human foetal shoulder joint. Cells, Tissues, Organs. 2002;170:1-20.
9. Moseley HF, Overgaard B. The anterior capsular mechanism in recurrent anterior dislocation of the shoulder- morphological and clinical studies with special reference to the glenoid labrum and the glenohumeral ligaments. The Journal of Bone and Joint Surgery. 1962;44B(4):913-27.
10. Sarrafian SK. Gross and functional anatomy of the shoulder. Clinc. Orthop. 1983;173:12-9.

11. Schmidt HM, Vahlensleck M. Clinicoradiologic anatomy of the shoulder region. Radiologc. 1996;36(12):933-43.
12. Hamilton, Boyd, Mossman. Human embryology. 4th ed. London: Williams and Wilkins company; 1976: 545.
13. Basmajian JV, Slonecker CE. Grant's Method of Anatomy. 11 ed. Baltimore: Williams and Wilkins. 1993: 374-379.
14. Prodromos CC, Ferry JA, Schiller AL, Zarins B. Histological studies of glenoid labrum from foetal life to old age. The Journal of Bone and Joint Surgery. 1990;72-A(9):1344-8.
15. Cooper DE, Arnoczky SP, O' Brien SJ et al. Anatomy, histology and vascularity of the glenoid labrum - an Anatomical study. Journal of Bone Joint Surgery. 1992;74(1):46-52.
16. Hube WP, Putz RV. Periarticular fibre system of shoulder joint. Arthroscopy. 1997;13:680-91.
17. Vangsness CT, Jorgenson SS, Waston T, Johnson DL. Origin of long head of biceps from scapula and glenoid labrum. Journal of Bone and Joint Surgery. 1994;76:951-4.

DOI: 10.5455/2320-6012.ijrms20140256
Cite this article as: Nazir S, Raina S, Bashir Z, Bano S, Shadad S, Khan JA, Jan Y. Histological development of human foetal shoulder joint. Int J Res Med Sci 2014;2:293-9.