ESTIMATION OF AGE OF OSSIFICATION OF HYOID BONE BY RADIOLOGICAL AND HISTOPATHOLOGICAL EXAMINATION OF AUTOPSY SPECIMEN

Dissertation submitted in partial fulfillment of the requirements for the Degree

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CERTIFICATE

This is to certify that this Dissertation entitled **"Estimation of Age of Ossification of Hyoid Bone by Radiological and Histopathological Examination of Autopsy Specimen"** is the Bonafide work of Dr.K.V.Vinoth, a postgraduate student at the Institute of Forensic Medicine, Madras Medical College from 2010-2013.

This Dissertation is submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the requirements for the MD degree in Forensic Medicine (Branch XIV).

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DECLARATION

I, Dr.K.V.Vinoth, solemnly declare that the dissertation titled **"Estimation of Age of Ossification of Hyoid Bone by Radiological and Histopathological Examination of Autopsy Specimen"** is the bonafide work done by me at the Institute of Forensic Medicine, Madras Medical College under the expert guidance and supervision of Capt. Dr. B.Santhakumar, Professor and Director of Institute of Forensic Medicine, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfilment of requirement for the award of M.D., Degree (Branch XIV) in Forensic Medicine.

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CONTENTS

INTRODUCTION

INTRODUCTION

Identification is essential in living persons, recently dead persons, decomposed bodies, mutilated bodies and skeletal remains. The main part of corpus delicti (i.e. the body of the offence; the essence of crime) is the establishment of the identity of the dead body.

The three primary characteristics of identification of a person are *Sex, Age and Stature*. Visual identification becomes difficult and impossible in cases of explosions, fires, advanced decomposition, mutilation, earthquakes, aircraft accidents, mass disasters and other terrorist activities.

During a person’s life, their bones would constantly undergo changes and those changes would follow a chronological pattern. Knowledge on those changes occurring in the bones would help in estimating age from the skeleton. The time of appearance of centers of ossification and the process of union of the epiphysis with the diaphysis have a sequence and time period, which is utilized towards age determination.

However, countable differences may be noticed in the appearance and fusion activities of the ossification centers depending on the sex, race and geological distribution. Ossification process may also be influenced by food, nutritional status, physical activity, metabolic and hormonal disturbances. Ossification activities occur earlier in Indian population than in Western population

Estimation of age after 25 years becomes more uncertain in living person and the dead[6, 23]. It is difficult to determine the accuracy of age after the full permanent dentition and fusion of all centres of ossification of long bones. The changes suggestive of advancing age in an individual were as follows: The fusion of the body with the greater cornu of the hyoid bone and the fusion of the manubrium and xiphisternum with the body of the sternum, the lipping of the vertebrae, fusion of exocranial and endocranial sutures of the skull bone, calcification changes of the cricoids and thyroid cartilages and Gastofson's method for estimating age from teeth [24]. All those mentioned changes may occur between 40 – 60 years.

The ossification changes resulting in fusion of greater cornu of the hyoid bone with its body occurs in a wide age groups. Trotter M[12] stated that fusion of greater cornu with the body of the hyoid bone occurred in middle age group and the ossification process may obliterate the joint space at later years. Fusion of the body with the greater cornu of the hyoid bone occurred as early as 18 years, and on other end, no fusion was found even in eighth and ninth decades. Indian authors like Parikh[1], Vij[23], Krishnan[25] had reported that 40-60 years was the age group at which greater cornu of the hyoid bone would unite with its body. The studies done abroad by Parson[26] and Miller[20] showed that greater cornu of the hyoid bone unites with its body at an earlier age group i.e. at 30- 40 years. Shimzu [21] DiMaoi [29] Nikolic[30] observed that as age increases the frequency of union of greater horn of the hyoid bone with its body was found to be increased. The age at which hyoid bone ossifies and their fractures were interrelated. Fracture was more frequent in persons over 40 years of age. The reason was that, after 40 years, the joint space between the greater horn of the hyoid bone with its body got ossified and thus the elasticity of the bone was lost making it susceptible to get fractured in case of external compression of the neck. Thus, it was stated that, susceptibility of the hyoid bone to get fractured was directly related to its age[18, 19]. The slope and curvature of the hyoid bone also contribute to its fracture in case of strangulation[8, 35].

It was of considerable interest for the forensic experts in estimating the age of an individual based on fusion of the greater horn of the hyoid bone with its body when partial unrecognized, decomposed bodies or skeletal remains were available. In the present study, radiological and histological analysis of ossification of greater horn of the hyoid bone with its body was done to narrow down the age at which it would ossify.

AIMS AND OBJECTIVES

- To estimate the age of fusion of greater horn of the hyoid bone with its body using radiological and histological examination.
- To find out the sexual variations in the fusion of greater horn of the hyoid bone with its body.

REVIEW OF LITERATURE

Age is a primary characteristic in the identification and its estimation is of considerable importance. The age of an individual especially in earlier years can be determined from 1) Physical examination 2) Dental examination 3) Ossification of bones. Physical examination for determining age is mainly based on appearance of secondary sexual characters. Teeth are useful for age determination from by the state of development and their secondary changes.

The bones of the human skeleton are preformed in hyaline cartilage. This soft tissue model is gradually converted into hard osseous tissue by the development of osteogenesis, frequently in a central portion from which the process of transformation spreads, until the whole skeletal elements are ossified. The appearance of such centres of ossification is spread over a long period of time, a large number are first detectable in the embryonic life, and some appear much later in prenatal life and others after birth. At the eleventh prenatal week, there are 806 ossification centres for growth and development of bone; at birth there were about 450 ossification centres, while the adult skeleton has 206 bones. This shows that 600 ossification centers were fused to give rise to adult bones. The process of appearance and union has a sequence and a time. As a rule, ossification begins centrally in epiphysis and spreads peripherally as it gets bigger.

Many bones are ossified from single centre, e.g. Carpal and tarsal bones. Most bones are ossified from several separate foci, one which appears near the middle of the future bone. This centre is concerned with progressive ossification towards the bone ends. In all such bones their ends are cartilaginous at birth. These terminal regions are ossified by separate centres, sometimes multiple; they are said to be called as secondary centres.

Krogman (1960) reviewed the reliability of the identification of the human skeletal remains. Bones being structures which form skeleton are made up of hard durable minerals and as such they resist decomposition for a longer period. Forensic study of bones gives information regarding age, sex, injury sustained by the person and some pathological information including those arise from chronic poisoning with heavy metals. Serological tests of bone give excellent evidence in DNA typing of an individual. The ossification changes of the hyoid bone helps in establishing the age of the individual after 25 years of age.

Analysis of all the possible age-related changes is the best for an overall estimate of the age of an individual. Some of them are:

1) General development, in case of children
2) Dental Eruption and Occlusion
3) Cortical Bone Histology

4) Cranial Suture Closures

5) Ossification of bones

6) Pubic Symphysial Face Morphology

7) Age-Related Degenerative Conditions

8) Phase Changes in the Sternal Rib

9) Secondary sexual characters.

1. General development

Gestational age of the fetus is calculated from the maturation of chorionic villi, length and weight of the fetus, head and chest circumferences, scalp hairs, closure of anterior fontanelle, nails, position of testes, foot length and ossification centers of the fetus.

In childhood, anthropometry, closure of anterior fontanelle, ossification centers and dentition are useful.

2. Dental Eruption and Occlusion

Scheuer and Black, 2000 and Smith, 1991 used dental eruption methods. While using dentition, age estimation was based on the eruption of the deciduous and permanent tooth. This method was useful up to about 15 years of age. Third permanent molar erupts after 15 years of age and if it didnt erupt, and then it cannot be reliable for determining age. Occlusal wear is also one of the indicators of age. But this is highly inaccurate.

The following characters are noted, while dentition is used for age estimation viz. teeth development and eruption. The alveolar cavities are formed at or around the third or fourth month of intrauterine life. Both the upper and lower jaw bones contained rudiments of all the temporary and first permanent molars at birth. In age estimation, teeth are useful by state of development i.e. temporary or deciduous or milk teeth and permanent teeth, and secondary changes. Gustafson's method is used for age estimation by analyzing the secondary changes of teeth like Attrition, Paradentosis, Secondary dentin, Cementum apposition, Root resorption and Transparency of root. The method to estimate age of dead infant is Boyde's method. In this method, daily incremental line in the enamel of teeth is used[5].

3. Cortical Bone Histology

Kerley (1965) was the first one, who published the technique of prediction of human skeletal age. He used histology of bone for age estimation, based on counting the number of osteons in bone bits taken from mid-shaft region of long bone sections. The samples were taken from the outer one third of the cortex. Ordinary light microscope was used and the variables were 100 x counted in four peripheral fields. A percentage assessment was estimated. These percentages were applied into regression formula or pre-determined age profile chart. Kerley concluded that, a reliability of almost 90% with standard deviation of +/- 5 years, with the good correlation from the fibula, then the femur and the tibia. He analyzed the variables like number of osteons, number of osteon fragments, and percentage of lamellar bone the percentage of circumferential lamellae cortex of bone. Singh and Gung (1970) analyzed the bone microscopy for various indicators including osteons and related components.

In these indicators, the number of osteon and osteon fragments were increasing with age. In the histologic method of age estimation, it was observed that size and shape of osteons increase with age. In contrast, the non haversian canals and percentage of circumferential lamellar bones are decreasing with age and they will disappear completely after fifty years of age. With the similar type of study, Rai et al had derived a regression equation for estimation of age. The equation is

$$Age = \text{number of osteons} + 8.3.$$

4. Cranial Suture Closures

This method is based upon the degree of closure, union or ossification of the cranial sutures in stipulated ages. Till recently, these methods have been considered inaccurate. But Meindel and Lovejoy (1985) had identified that closure of parietal ectocranial sutures was a significant indicator of age above 40 years. Mann et al. (1987) had stated the four maxillary sutures and their patterns of closure could be used as a reliable age estimator.

4. Ossification of bones

Krogman and Iscan1986 and Stevenson 1924, used epiphyseal closure and ossification methods for age estimation. Cartilaginous bridges between the growing bones form the endochondral bones of postcranium by union and ossification. This process used to follow a pattern of growth algorithm. It is also used to assess age at death on the union/non-union basis. McKern and Stewart have defined five grades of epiphyseal union: unobservable (0), beginning (1), active (2), recent (3), and complete (4). These can give a possible accuracy estimation of age.

6. Pubic Symphyseal Face Morphology

In the young age, the pubic symphyseal face is categorized by an undulating surface of typical non-fused epiphyseal plate. From age 18 onwards, this surface undergoes continuous progressive metamorphosis. With this character, Suchey and Brooks have studied age estimation with the male pubic symphysis.

7. Age-Related Degenerative Conditions

The osteophytic growths form on the outer margins of the vertebral body. Steward has computed an age evolution histogram over 21 years based on the percentage of extra lipping as a function of age for the dorsal and lumbar vertebrae.

8. Phase Changes in the Sternal Ribs

Iscan and Loth have established a method of age estimation based on observable changes at the sternal end of the fourth rib. They are parallel to those that occur on the pubic symphyseal face. They are of exact morphological nature and occur on the costochondral joint between rib and sternum. The variables are as follows: an upsurge in the depth of the articular depression and the degenerative fragmentation, thinning and augmented porosity at the edges of the articular surface over the period of time.

9. Secondary sexual characters

While using secondary sexual characters, to assess the age in both gender, mustache, axillary, and pubic hair are used. In males, position of testes also will be used for age estimation. In females, menstruation, breasts changes and external genitalia changes are useful.

Factors affecting the bone growth and development:

A number of factors influence bone growth and development. These include nutrition, hormonal secretions, physical exercise and exposure to sunlight.

Vitamin A and C are required for normal bone growth and development. Vitamin A is essential for osteoblast and osteoclast activity during normal bone development. Vitamin C is needed for collagen synthesis and its deficiency may inhibit bone development. Thyroid hormone stimulates replacement of cartilage in epiphyseal plate of long bones. Thyroid hormone will cause premature ossification of the epiphyseal plate inturn arresting the growth of the bone. Parathormone stimulates an upsurge in the number and activity of osteoclast.

Anatomy of the hyoid bone:

The hyoid bone is U- shaped. It develops from the second and third branchial arches. It is positioned in the anterior part of midline of the neck between the chin and the thyroid cartilage and it lies at the level of third cervical vertebra.

The hyoid bone consists of a central part called the body and two pairs of greater and lesser cornua[2]. The hyoid bone does not articulate with any other bone, but suspended by muscles and ligaments (Fig: 1). The major function of these muscles is to aid in swallowing and speaking[17].

The body of the hyoid bone is irregular, elongated and quadrilateral. Its anterior surface is convex, faces anterosuperiorly, and is crossed by a transverse ridge with a slight downward convexity. The posterior surface is smooth, concave and faces posteroinferiorly[3]. Epiglottis is separated from the posterior surface of the hyoid bone by the thyrohyoid membrane. The body is divided by a median bridge into halves.

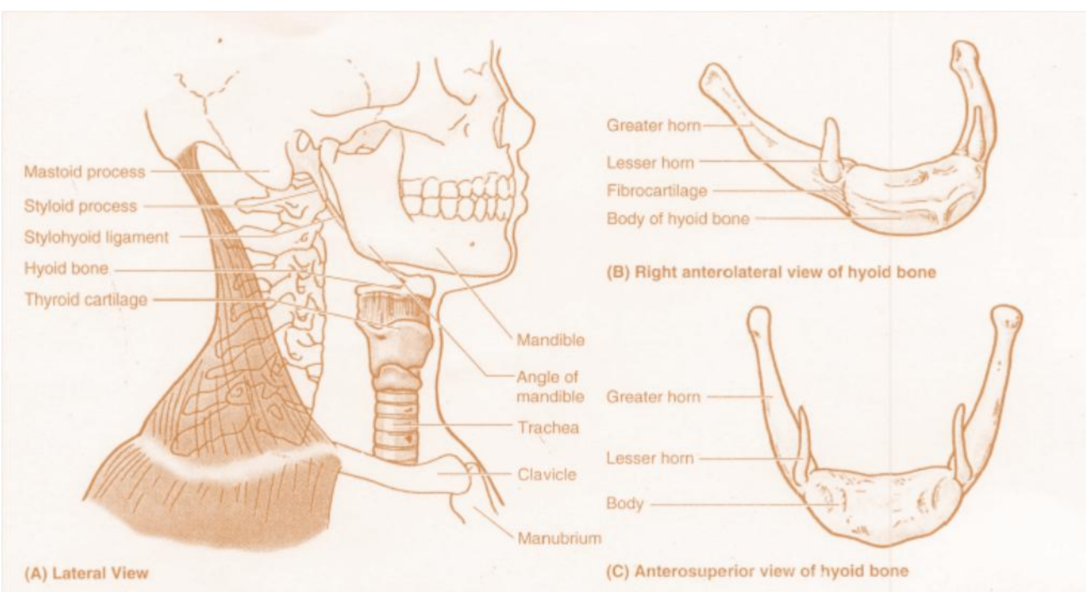


Fig: 1 Anatomical position and anterolateral and superior view of the hyoid bone.

In early life, greater horn of the hyoid bone is connected to its body by cartilage, but after middle age they are usually united by bone. They project backwards (curving posterolaterally) from the lateral ends of the body. They are horizontally flattened, taper posteriorly, and each ends in a tubercle. When the throat is gripped between finger and thumb above the thyroid cartilage, the greater cornua can be identified and the bone can be moved from side to side. The middle pharyngeal constrictor and more laterally (i.e. superficially) hyoglossus, are attached along the whole length of the upper surface of each greater cornu.

At the place where the greater horn and body meet are the two small conical projections known as lesser cornua. A fibrous tissue connects the base of lesser horn with the body. Occasionally, lesser horn will be united to the greater horn by a synovial joint which occasionally becomes ankylosed. The middle pharyngeal constrictors are attached to the posterior and lateral aspects of the lesser cornua. The stylohyoid ligaments are attached to their apices and are often partly calcified.

During swallowing, hyoid bone acts as a fulcrum for the muscles which takes part in swallowing. Suprahyoid muscle elevates the hyoid bone when the mandible is fixed.

Embryology and Formation of Bone:

During embryogenesis, bone tissue arises by two processes: intramembranous ossification and endochondral ossification. In endochondral ossification, a cartilage model of the bone is first formed and it is later replaced by bone. The weight-bearing bones of the axial skeleton and the bones of the extremities develop in this manner. The first bone to arise, whether from mesenchyme or from cartilage is in the form of spicules. These spicules are made of immature bone, known as woven bone.

In immature bone, the collagenous lamellae are not arranged in parallel or concentric arrays as in mature spongy and compact bone respectively, but they are randomly oriented and loosely intertwined and hence called as woven. Immature bone also has more ground substance than mature bone. Consequently, immature and mature bone show different staining characteristics, immature bone stains more with haematoxylin and mature bone stains more with eosin.

The spicules of immature bone are remodelled. The remodelling process can give rise to more spongy bone or compact bone. The remodelling of bone continues throughout the life. In the developing foetus, it is the immature bone which is predominant. In the adult, most immature bone is replaced by mature bone, but immature bone is seen in places where bone is being remodelled or repaired, and in certain specific areas, such as the alveolar sockets of the oral cavity. When bone matrix is first secreted, it is not yet mineralized and is called osteoid.

Intramembranous ossification:

In this method, bone development is not preceded through cartilage model. But it develops from mesenchyme. The mesenchymal cells directly differentiate into osteoblasts. Then osteocytes and osteoclasts are developed. The clavicle, flat bones of cranial vault, mandible and maxilla are examples of intramembranous ossification. This process also contribute thickening of long bones. Intramembranous ossification takes place within the condensation of the Mesenchyaml tissues. It is the source of the flat bones and it is responsible for the growth of the short bone.

The osteoblasts maintain contact with one another via cell process. The osteoid becomes calcified with time, and osteocytes will be become enclosed in canaliculi. Some of the mesenchymal cells surrounding the developing bone spicules proliferate and differentiate into osteoprogenitor cells. Osteoprogenitor cells which in contact with bone spicule become osteoblasts, and secrete matrix, which results in appositional growth of the spicule[7]. Intramembranous ossification begins at about the eighth week in the human embryo.

Endochondral Ossification:

In human body, enchondral ossification begins in the second trimester and continues upto early adulthood. Many of the bones, in human, are produced through this method. This form of ossification is responsible for formation of short and long bones and is taken place in hyaline cartilage which already resembles a small version of the fully grown bone. This process starts with a structure known as bone collar that is produced by the process of intramembranous ossification within the local perichondrium. During the process blood vessels, penetrate through the bone collar and transfer the osteoprogenitor cells in this region. The bone collar or cartilage model proceeds to develop by both the appositional and interstitial means. The bone collar is merely bone tissues appearing a shallow bone cylinder surrounding the mid portion of the cartilage. Then the chondrocytes divide, enlarge, mature and cartilage begins to calcify. Now the perichondrium ultimately becomes the periosteum. While the calcification proceeds, the diffusion of nutritional particles and gaseous materials through the calcified matrix decreases. The characteristics of the step include cell enlargement, matrix calcification and cell death. Following death of chondrocytes, the small pieces of calcified matrix serves as framework for deposition of bony materials. The result of these processes is an area of three dimensional structures formed by the remnants of the calcified cartilage matrix**.** Subsequently the inner perichondrial exhibit their osteogenic potentials. A thin periosteal collar of bone forms around mid-shaft region of bone. Then osteoprogenitor cells, osteoblasts, osteocytes and osteoclasts develop. Mesenchymal tissues, blood vessels and osteoblasts form primary ossification centre at the diaphyseal region of the bone and secondary ossification centre on the epiphyseal region of the bone[37]. Secondary ossification centers form at the swellings in the extremities and together with the primary ossification centers eventually form cavities. These cavities gradually fill with bone marrow. The epiphyseal cartilage is responsible for the growth in length and when the epiphysis closes the cessation of the bone growth.

Epiphyseal cartilage can be divided in to seven zones:

1. Resting zone
2. Zone of proliferation
3. Hypertrophic zone
4. Zone of calcification
5. Zone of retrogression
6. Ossification zone
7. Resorption zone

Resting zone is otherwise known as the quiescent zone. And it is mainly composed of hyaline cartilage and is present in the nearest to the end of the bone.

As ossification approaches this initial short zone progressively shorten and generally slow the growth in all direction. The next zone is an active zone and involves the cells of the resting zone producing daughter cells. It aligns themselves in distinct columns parallel to the long axis of the bone. Each row of cells grow by the addition of the more cells and so forth. The mechanism of the zone allows the cartilage to increase in length. The third zone involves the maturation of the cells in which large lacunae can be found with thin adjoining septae. The next zone is involving the process of calcification of the matrix surrounding the enlarged lacunae. Following calcification there is zone of ossification. Here the osteoblasts differentiate from the mesenchymal cells and gather the exposed plates of calcified cartilage. Here osteoblasts cells lay down the bone at this stage, and enchondral bone tissues appears. The last stage is known as the resorption zone. Here the resorption of the bone in the center of the diaphysis takes place resulting an increase in size of the marrow cavity.

Embryology of hyoid bone:

The hyoid bone develops from cartilages of second and third pharyngeal arches. Chondrification begins in the fifth week of intra uterine life and gets completed in the thirs and fourth month of intrauterine life.

Primary ossification proceeds from six centres. Two ossification centre for the body, two for greater cornu and two for lesser cornu. Ossification starts in the greater cornu towards the end of intrauterine life and in lesser cornu around puberty.

BONE HISTOLOGY

Bone is the main constituent of adult skeleton. It supports fleshy structures, protects vtal organs such as those present in cranial and thoracic cavity. Bones is a special form of connective tissue and comprises three types of cells nalmely osteoblst, osteocyte and osteeoclast and predominantly collagenous extracellular matrix. The matrix is made up of type I collagen and unminerilised matrix is known as osteoid. The deposition of calcium hydroxyapetite in the osteoid results mineralization of matrix, thus imparting considerable rigidity and strength to the bone.

Osteocytes are present in the laculane of the matrix. Organic components of the matrix are synthesized by the osteoblast. Osteoclast are multinucleated giant cells that are involved in bone resorption and bone remodeling[4].

Bone acts as a reservoir of calcium, phosphate and other ions. These ions can be released or stored in a controlled way thus maintaining constant concentration of ions in the body fluids. Canaliculi are thin, cylindrical spaces which perforate the bone matrix. Through these canaliculi, exchange of metabolites takes place between the osteocytes and the blood capillaries. Bones are lined by layers of tissue containing osteogenic cells; they are endosteum on the inner surface and periosteum on the outer surface of the bone

Due to the hardness of the bone sectioning by using microtome is difficult. For this special techniques are used, that permits observation of organic matrix and cells after decalcification of the bone preserved by standard fixatives.

Types of bone:

Histologically bones are classified as mature bone and immature bone. Mature bone is further divided into compact bone and spongy bone. Compact bone is also known as dense bone or cortical bone. Spongy bone is also termed as cancellous bone, trabecullar or medullary bone. Compact bone and spongy bone are located in specific areas. In long bones, most part of the diaphysis is made of compact bone with small portion of spongy bone facing the marrow cavity. The epiphysis of the long bone consist of spongy bone covered with a shell of compact bone. The flat bones of the skull have a middle layer of spongy bone placed between two thick layers of compact bone.

Compact bone:

Compact bone is made up of hard and dense material. It is vascular in nature. It has uniform smooth structure. It is situated in cylindrical outer part or shaft region of the long bone. In compact bone, the collagen fibres are arranged in thin layers of bone[7]. Most of the compact bone consists of osteons. When we examine a section of compact bone, we find that this type of bone is made up of lamellae, and is pervaded by lacunae, and by canaliculi.

Cancellous bone:

Cancellous bone is not dense like compact bone. It is situated inner to the compact bone and adjacent to the bone marrow cavity. It contains numerous interconnecting spaces. If we examined the ends of the bone, we can see the meshwork of tiny rods or plates of bone called **trabeculae** contained numerous space appeared like that of a sponge. So it is also called as spongy bone[36]

In microscopic examination, there are two varieties showed. They are **primary, immature** or **woven bone** and **secondary, mature** or **lamellar bone.**

Primary bone tissues are usually temporary in the body. These are newly formed bones. These newly formed bones do not have lamellae. In these varieties, the collagen fibres were found randomly interlacing each other. These are woven bone. Except near the cranial sutures, tendon insertions and tooth sockets, they are replaced by lamellar Secondary bone or secondary bone tissues, in future, in adults.

In contrast, secondary bone tissues are usually found in adults. It characteristically showed the lamellae. If the bone is made up of layers or **lamellae**, then it is called as **lamellar bone**. Lamellae are of two types. **Interstitial lamellae** are present in the angular intervals, between the adjacent osteons. These are remnants of osteons. **Circumferential lamellae** are arranged near and parallel to the surface of compact bone. Lamella is thin plate of bone consisting of collagen fibres and mineral salts that are deposited in a gelatinous ground substance. Lamellae are 3 – 7 micrometer thick[38]. They are parallel to each other in periphery of the bone or they are mostly arranged as concentric rings around a blood vessel. The outer circumferential lamellae are deep to the periosteum. The inner circumferential lamellae surround the marrow cavity.

Significance of hyoid bone in Forensic Medicine:

Significance of the hyoid bone in Forensic Medicine is in relation to criminal cases of assault involving manual strangulation (throttling) where the bone may get fractured. The hyoid bone may or may not get fractured during strangulation and it is related to the intrinsic anatomic features of the hyoid bone. The hyoid bone fracture occur more commonly in older victims of strangulation

(39 ±14) years) when compared to the victims with unfractured hyoid bones (30 ± 10 years). The age- dependency of the hyoid bone fracture also correlates with the degree of ossification of the hyoid bone.

The shape of the hyoid bone was also found to influence the fracture of the hyoid. Fractured hyoids were longer in the anterioposterior plane and were more steeply sloping when compared with unfractured hyoid. Therefore, the fracture of hyoid bone in victims of strangulation would depend on the age of the victim, the ossification process and the shape of the bone.

The fractures to the hyoid bone classified into three groups:

1. Inward compression fractures
2. Anteroposterior compression fractures
3. Avulsion fractures.

1. Inward Compression Fractures:

This type of fracture occurs in throttling, where the main force is inward compression acting on the hyoid bone. Here the fingers of the grasping hand squeeze the greater horns towards each other, due to which the bone may be fractured and the posterior fragment is displaced inwards. The periosteum is torn on the outer side of the bone, but not on the inner side.

2. Anteroposterior Compression Fractures:

In cases of hanging, the hyoid bone is forced directly backwards due to which the divergence of greater horns is increased which may cause fracture with outward displacement of the posterior small fragment. In such a case, the periosteum is torn on the inner side of the fracture only, due to which the fragment can be easily moved outwards, but inward movement is limited to the normal position only.

3. Avulsion fractures:

They occur due to muscular over activity, without there being any direct injury to the hyoid bone. They are also called tug or traction factures.

The steps involved in processing of bone for histological analysis are:

1. Fixation: With 10 % neutral buffered formalin
2. Decalcification with 5 % Nitric acid
3. Embedding with wax
4. Section cutting with microtome
5. Staining with H and E
6. Mounting with DPX

Fixation is the important step in obtaining a good histological specimen. The important solutions employed for fixation include 10–30% formalin, paraformaldehyde, glutaraldehyde, and alcohol-based solutions. The standard solution is 10% neutral buffered formalin (NBF). Specimens should be placed in buffered formalin for 12 to 24 hours. There should be 10:1 ratio of fixative to tissue. To enhance fixation, agitation of the specimen in fixation can be helpful.

Decalcification of bone:

Decalcification is the removal of the inorganic components from the hard tissue. The usual mechanism for decalcification is immersion in an acid solution, a chelating solution, or a solution consisting of an acid-chelator complex. This immersion may be augmented by heat, vacuum, or agitation, so as to decrease the time for complete calcium removal.

Acid solutions will destroy the organic substances of the bone tissue; therefore, it should be adequately protected before decalcifying it. For that, fixation in neutral buffered formalin (NBF) is done. The longer the duration the block kept in formalin, the greater will be the resistance of the nucleic acid to hydrolytic action of decalcifying acid. Nitric acid decalcification is the fastest but the end point has to be carefully watched for; otherwise, progressive tissue damage occurs and staining is severely impaired[10].

Test for decalcification

Decalcification can be tested in a number of ways such as by touch, pliability and resistance to finger nails, by needling, by X - ray or by chemical testing of the decalcifying fluid. The physical methods are not recommended as they damage the tissue. X-ray will be good but not always convenient. Chemical test is favored. In a clean test tube, 5ml of decalcifying fluid is placed which is kept in contact with the bone block for 3-12 hours. Alkalinization of the litmus paper by addition of strong ammonia and water and shake i/.t after each addition.5ml of saturated aqueous ammonium oxalate is then added and mixed and made it to stand for 15 -30 minutes. If fluid remains clear, the process of decalcification is complete. If cloudiness develops it is due to the calcium oxalate and means that decalcification is not complete.

The further steps for processing in the decalcified tissue include dehydration, clearing in xylene, infiltration and embedding. Ethanol is the most commonly used dehydrating agent. Dehydration is carried out in stages using increasing strength of alcohol. Clearing is the replacement of dehydration fluid in the specimen with a substance that is miscible with the embedding medium to be employed. Infiltration displaces the clearing agent from the tissues and allows the tissue to be completely permeated by the paraffin, which is then subsequently allowed to harden (embedding), thereby producing a block from which sections can be cut. Following infiltration of specimen with paraffin, the specimen is transferred into a mold and fresh wax added, and then the molten wax containing the specimen is allowed to harden. Paraffin is most suitable for embedding decalcified hard tissues for thin sections of 3–6□m, and is the most widely used embedding method. For section cutting, rotary microtome are used to cut 6–10-µm-thick sections. After satisfactory sections are made, they are transferred into a flotation bath using a jewelry forceps or a fine brush for flattening and for mounting onto microslides. The most commonly used reagents for staining are hemotoxylin and eosin. The slides are mounted with D.P.X mountant.

Previous studies

Kaur Harjeet et.al[11] studied *"Time of fusion of greater cornu with body of hyoid bone in Northwest Indians"*. A sample of 200 hyoid bones were collected from the cadavers on whom medicolegal autopsy had been performed. Out of which 133 were males and 67 were females. The hyoid bones were freed from the muscles and soft tissues of the laryynx and the tongue; adherent soft tissues were removed carefully and preserved in 10% formalin. The hyoid bones with displaced joint between the greater cornu and the body of hyoid bone, fracture of hyoid bone was not included in the this study. Hyoid bones were examined radiologically by dental occlusal film. Antero-posterior view was taken to study the fusion of body with the greater cornu of hyoid.

Classification of degree of fusion was done; they were as follows :

i) Non fusion.

ii) Commencement of fusion.

iii) Partial fusion.

iv) Complete fusion.

O'Halloran et.al (1987)[16] studied ossification and age of hyoid bone between 1982-1986 at the Oregon state medical examiner office California. In this study, 300 hyoid bones were collected from the autopsy cases of age group between 2months to 92 years. In this study one third of the samples were examined after exposure to a colony of feeding dermestid beetles. Two thirds of the cases were radiologically examined. Fusion of lesser cornu with the body of hyoid bone was not appreciated in the X-ray film. Data obtained was correlated with the age, sex and racial data from the population group. For easy analysis age groups were divided into groups by decades. Out of this 45% of the male joints and 41% of the female joints showed some degree of osseous fusion.

Osseous fusion was not found until third decade. The incidence of fusion of greater cornu with body of hyoid bone increased with age and reached a plateau in sixth to seventh decade with approximately 70% of the joints in men and 60% of the joints in women fused by the age of 60 years. Of the total 300 hyoid bones, 89% (269) were Caucasians, 6% (18) black, 4% (12) oriental and 1% (3) was unknown. Bilateral fusion of the greater cornual joints was more frequent in men than in women in all age groups after the third decade fusion of the greater corneal joints at the time of death. There was no statistically significant interracial difference in greater cornu fusion rates to either males or females were found.

Miller K.W et al (1998) studied *"Age and sex-related variation in hyoid bone morphology"*. Hyoid bones collected during autopsy were examined during the period between 1987 to 1997 at the Ventura country, California. In this study 315 hyoid bones were examined radiologically. Each hyoid bone was radiographed with its inferior surface resting directly on the cassette 127 from the X- ray source with exposure of 50kVp and 1.25 mAs[20].

The fusion of lesser cornu with the body of hyoid bone was not included in this study due to difficulty in visualizing the joint. The samples were divided in the frequency of decade. Bilateral non fusions persist in a significant proportion of the elderly population. Bilateral non fusion was present in 30% of the individuals in this study who were 70 years of age or more. It was concluded that fusion of greater cornu with body of hyoid bone is common during the third decade with 13% of the individuals in this age group had unilateral fusion and 17% had bilateral fusion. In this study, it was stated that as age increases there was an increase in the frequency of fusion of greater cornu with body of hyoid bone.

A proportion of hyoid bones with bilateral non fusion remain relatively constant at about 30% after the fourth decade of life. No significant sex difference in the fusion of greater cornu with the body of hyoid bone was noted. The proportion of the males and females with bilateral non fusion is nearly equal and remains more or less constant after the fifth decade of life.

In the study done by **Kaur Harjeet et.al (2010)** it was stated that commencement of fusion indicates fusion had started just in the middle or one side and contact area of the fusion is less than one fourth[11].

Partial fusion represents fusion was advanced to involve more than half of the contact region.

Complete fusion represents more than 3/4th of the contact area was fused. Data was classified into five year intervals for statistical purpose.

In males it was found that non fusion on both sides of the hyoid bone between the greater horn and body was seen in 69 cases (51.9%), unilateral non fusion was seen in 23 (8.6%) cases. Bilateral complete fusion was observed in 22 (16.5%) cases and unilateral complete fusion was observed in 16 cases (6.0%). The remaining hyoid bones had other grades of fusion i.e. they may shoe either commencement of fusion or partial fusion.

In hyoid bones of less than 25 years of age non fusion of greater cornu with the body of hyoid bone was seen. Commencement of fusion in the age group of 26-30 years on both sides was seen in 4 (14.8%) cases. Bilateral complete fusion in that age group was seen in 3 cases. Unilateral fusion in that age group was seen in 3 cases (10%).

In the age group of 41-45 years, 7 subjects were included out of this bilateral complete fusion was seen in 3(42.9%) cases. Two cases (28.6%) showed non fusion.

Unilateral and bilateral fusion of the greater horn and body of the hyoid bone was seen in 8 cases (6%) and 37 cases (55.2%) of the total female cases in this study. It was stated that unilateral and bilateral fusion of the hyoid bone occurs in the same age group of 26-30 years in both sexes.

D'Souza et al studied hyoid bone fusion and its usefulness and impactions. In this study 130 hyoid bones were collected in the age group of 4-70 years. Fusion or non fusion of the body of hyoid bone with greater cornu of hyoid bone was observed radiologically. The earliest age in whom hyoid bone showed fusion was 20 years. Only one case showed complete fusion in this age. Even after 60 years of age it was found that non fusion was seen among the cases. Unilateral fusion was seen in one case after 60 years. It was stated that mean age of unilateral fusion and bilateral fusion in males was 39 years and 41 years respectively. In females, the mean age of unilateral and bilateral fusion was 37.5 and 45 years, respectively.

Bilateral non fusion of greater cornu with body of hyoid bone was seen in 23% of specimens aged above 40 years. In both sexes, 42 years was the mean age of bilateral fusion (Table: 1) No significant differences between males and females were found both in unilateral and bilateral fusion.

Table: 1 Mean age ± SD of fusion in the hyoid bone

GRADE	MALES	FEMALES	BOTH SEXES
Bilateral non fusion	27±11.56	23±7	25±10
Unilateral fusion	39±10	37.5±15	38±12
Bilateral fusion	41±11	45±12	42±11

Statistically no significant difference in the fusion of greater horn with the body of the hyoid bone between right and left side in both sexes. It was concluded that even though there was significant association between fusion of the hyoid bone with advancing age it cannot be considered as a good indicator for age estimation.

Shimizu et.al studied *"Age-related morphological changes in the human hyoid bone"*. This study was conducted in Sendai- Japan. In this study 32 measurements were done on the radiographs of 238 hyoid bones from autopsy cases whose age and sex was known.31 hyoid bones which were studied by radiography were also examined histologically in horizontal sections[21].

Analysis of the length and width of the hyoid bone revealed significant increase in the body and the anterior part of the greater cornu and a significant decrease in the posterior part of the greater cornu with aging. Most measurements of the body and the greater cornu revealed differences between male and female hyoid bones. There was a significant age related decrease in the breath of the joint space. It was found that frequency of fusion increases as the age increases.

Histological findings showed ossified or calcified fusion, with osteoclasts in the marginal area of the joint space. Increasing age induces fusion of the body and the greater cornu. The morphometric changes in the shape of the hyoid bone may represent functional adaptation to articulation fixation.

Atul gupta et al (2008) studied **"**Stage of fusion of hyoid bone**"**. In this study 170 hyoid bones were collected from the autopsy cases of age group 20–65 years, which includes 71 females and 99 males[22]. The aim of the study was:

i) To estimate the age of fusion of greater horn with body of the hyoid bone

ii) To find out difference in fusion of greater cornu with the body of hyoid bone in male and females

iii) To find out difference in fusion of greater cornu with the body of hyoid bone on right and left side.

The hyoid bone was kept in a wooden box and buried in the land for one month; this would remove the adherent soft tissues. The fusion of greater horn with the body of hyoid bone was studied and data was analysed statistically.

Samples were grouped into five year age intervals. To facilitate detail study of the fusion of the age groups larger intervals were avoided. To study the age of ossification of greater horn with the body of hyoid bone, Chi square test was done. Out of total 71 females, bilateral non fusion was seen in 38 females (53.52%), right side fusion was seen in 4 (5.6%) cases, left side fusion was seen in 4 (5.6%) cases and bilateral fusion was seen in 25 cases (35.21%). Non fusion was seen in none of the three cases in the age group between 20-25 years. 7 cases were present in the age group between 25-30 years, out of these cases, 6 cases showed bilateral non fusion and only one case showed unilateral fusion on right side.

8 cases were present in the age group between 30-35 years. Out of 8 cases, 5 cases(27.8%) showed bilateral fusion and 3 cases(16.6%) showed unilateral fusion. 8 cases were present in the age group of 35- 40 years; among this 6 (75%)

cases showed bilateral non fusion. Similarly, 5 (71.4%) cases showed bilateral non fusion out of total 7 cases in the age group of 40-45 years. Bilateral fusion was observed in 33% of total cases in the age group between 45- 50 years. After 50 years of age there was increase in the frequency of the fusion of greater horn with the body of the hyoid bone. All the 3 cases in the age group of 60-65 years showed complete fusion on both sides.

Out of total 99 male cases, bilateral non fusion was observed in 49 (49.5%) cases, unilateral fusion on right side was seen in 10 (10.1%) cases, and on left side was seen in 10 (10.1%) cases and bilateral complete fusion was observed in 30 (30.3%) cases. In this study, statistically significant association was observed between the age and ossification of hyoid bone. In both sexes, ossification of the hyoid bone increases with age. It was concluded in this study that fusion of greater horn and the body of the hyoid bone occur in the age group between 30-40 years and fusion occurred earlier in females about 5 years.

Partha pratim studied "*Morphometric features and sexual dimorphism of adult hyoid bone: A population specific study with forensic implications".* In this study 144 intact hyoid bones were studied by direct measurements taken by quality divider and metallic graduated scale with readings up to 1mm. It was concluded that less than 13 years sex difference in hyoid bone was insignificant[33]. The hyoid bone in the adult male were wider and longer than females. There was no stastistically signicance found in lengths of greater and lesser cornu and no significant difference in length of corny between males and females[33]

Vanejis et.al studied that incomplete fusion of greater horn with the body of hyoid bone is common and variable[31]. **Kim Deog-Im et.all** studied sex difference among Korean population by using digital photographs and concluded that accuracy of discriminants functions was 88.2% in both groups (statistically significant)[34].

Hiroyasu Kanetaka et.al (2011), studied *"Synostosis of the joint between the body and greater cornu of the human hyoid bone"*. In the study 259 hyoid bones were collected from the cadavers from the age group 16-98 years. The structure of the joint between the body and the greater cornu in the hyoid bone was examined histologically. Joints were classified into three grades based on histological observations.

Grade I showed fibro cartilage without degenerative change in the marginal region of the joint.

Grade II showed prominent calcification or ossification on the outer margin of the joint without fusion.

Grade III showed bony fusion.

Histological changes with age were revealed by a comparison of the prevalence of these three grades among individuals of three age groups: young adult (16-39 years), middle aged (40-69 years) and elderly (70+ years). Clefts with necrotic tissue were observed in cartilage along with progressive calcification. Calcification and ossification of joints were induced with age from fibrous tissue and cartilage on the outer margin of a joint. The authors suggest that the age changes in the joint between the body and greater cornu of the hyoid bone may affect the mobility of this joint and may be related to masticatory and swallowing functions[32].

MATERIALS AND METHODS

The present study "Estimation of age of ossification of hyoid bone by radiological and histopathological examination of autopsy specimen" had been carried out in the Institute of Forensic Medicine, Madras Medical College, Chennai, during the period of 2011 to 2012. Of all the cases brought to the institute for medicolegal autopsy, a sum total of 155 cases were selected randomly for this prospective study. Permission of the ethical committee on the use of human material for research purpose was obtained.

Dissection techniques:

For bloodless dissection of the neck first the brain and the thoraco abdominal contents were removed before proceeding to the neck dissection. A wooden block 12 to 20cm high was placed under the shoulders to allow the head to fall back and thus the neck was extended. A vertical incision was then made from the chin down up to the suprasternal notch. The skin over the region of the neck was held with a toothed forceps and subcutaneous dissection was carried to the lower border of lower jaw, laterally on the sides of neck and clavicle. Deep cervical fascia was reflected from cervical muscles and strap muscles of the neck were exposed, inspected and reflected on each side. Thyroid gland and carotid sheath was freed by blunt dissection.

Larynx, trachea, pharynx and oesophagus were mobilized and pulled away from the prevertebral tissue by blunt dissection. The mouth was opened and the tip of tongue pushed upwards and backwards. The scalpel was inserted under the chin through the floor of the mouth and cut along the sides of the mandible to the angle of the mandible dividing the neck muscles attached to the lower jaw. At the angle of mandible, blade was turned inwards and tongue was pushed down under the mandibular arch, soft palate was cut to include uvula and tonsils with the tongue and the neck structures were removed en masse.

Posteriorly, the attachments were freed from the prevertebral muscles on the anterior surface of the cervical vertebra till the jugular notch and the great vessels were divided in the neck.

Hyoid bone was freed from the surrounding soft tissue. Due care was taken not to break the hyoid bone during the removal. It should be noted that the normal joint mobility between greater cornu and body of the hyoid bone should not be confused for the fracture of the hyoid bone[27]. Mobility of greater cornu with the body of hyoid bone was noted. A specific numbered tag was tied to the dissected hyoid bone specimen for which postmortem number, age and sex were noted separately and preserved in 10% formalin solution. X Rays of the collected hyoid bones were taken. An individual separate token number made of lead was placed above the individual hyoid bone specimen (for better visualization in X-ray film). A group of 16 hyoid bones were taken by a single exposure of the X-ray beam.

As per the observations and analysis of previous studies based on radiological assessment of degree of fusion of hyoid bone, in this study, antero-posterior view was taken to study the fusion of body with the greater cornu of the bone.

Radiologically degree of fusion of body and greater cornu of the hyoid bone was classified as:

i) Non fusion

ii) Commencement of fusion

iii) Partial fusion and

iv) Complete fusion.

Commencement of fusion indicates fusion had just started either in middle or at one side and fusion of contact area was less than one fourth.

Partial fusion indicates that fusion had advanced to involve more than half of the contact area and fusion did not exceed three fourth of the contact area. Complete fusion indicates that more than 3/4th of contact area was fused.

Based on the above grades of fusion of body with the greater cornu of hyoid the bone, each radiological film of the hyoid bone was studied and the results were tabulated.

After radiological examination of the hyoid bones, junction of greater cornu and body of hyoid bone on one side was cut and the bits were preserved in 10% formalin and separate label was attached to the container mentioning postmortem number, age and sex. Bones were decalcified, processed and stained with Hemotoxylin and Eosin and read under low power of the light microscope. The structure of the joint between the body and the greater cornu in the hyoid bone was examined under the microscope.

The ossification between the body and greater corner of the hyoid bone are classified into three grades based on histological observations.

Grade I showed fibro cartilage without degenerative change in the marginal region of the joint.

Grade II showed prominent calcification or ossification on the outer margin of the joint without fusion.

Grade III showed bony fusion.

Results were tabulated.

RESULTS

In the present study 155 hyoid bones were collected from the autopsy cases submitted to the Institute of Forensic Medicine, Madras Medical College during the period of 2011- 2012. The samples were collected by ***Simple Random Analysis.*** Out of 155 hyoid bones that were collected, 133 belonged to males and 22 hyoid bones belonged to females (Fig: 2)

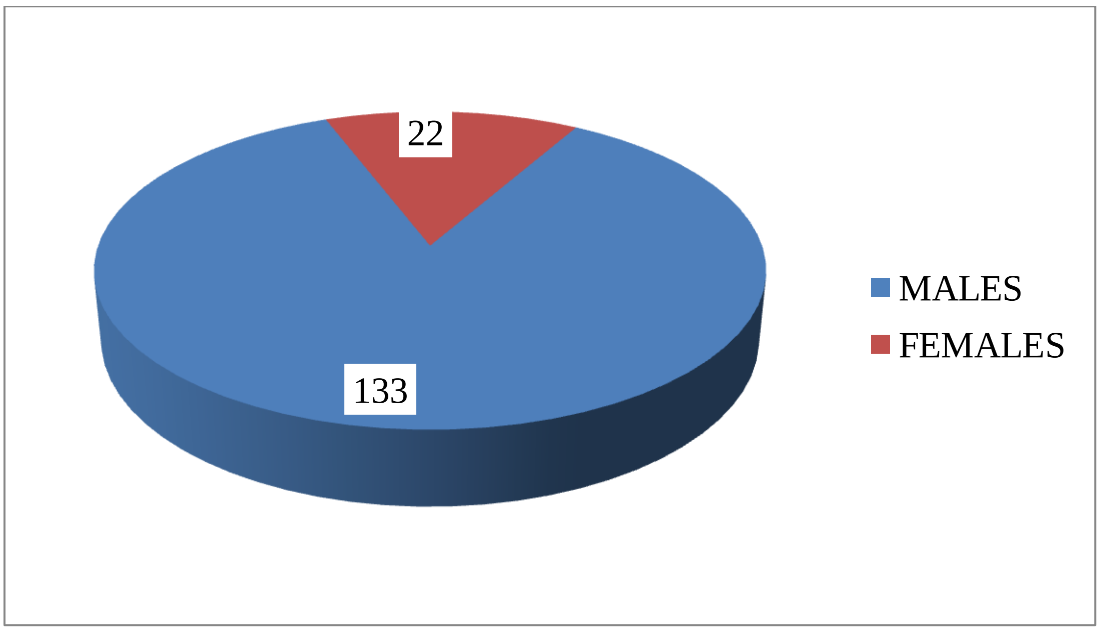


Fig: 2 Number of samples of hyoid bone among male and female.

Samples were categorized into five year age intervals for statistical analysis. So the age groups between 30 – 60 years were grouped into 6 intervals.

In the age group of 30 -35 years, a total of 42 hyoid bones were collected; out of which 38 were males and 4 were females. In the age group of 36- 40 years 44 hyoid bones were present which comprises of 36 males and 8 females. In the age group of 41- 45 years 27 cases were analyzed, out of which 22 were males and 5 were females. In the age group of 46-50 years, 16 were males and 2 were females. In the age group of 51-55 years 18 cases, this includes 17 males and 1 female. In the age group of 56-60 years 6 hyoid bones were analyzed, out of which 2 were female and 4, were male.

Non fusion:

30 – 35 years:

In the first age group i.e. between 30 – 35 years, 9 cases (21.4 % within that age group) showed unilateral non fusion and 33 cases (78.6%) showed bilateral non fusion (Table: 2). All the 9 cases of unilateral non fusion were of males and among 33 cases of bilateral non fusion 29 cases belonged to male and 4 cases belonged to female.

Table: 2 Non fusion among both sexes

AGE GROUP	BOTH SEXES	OTHER GRADES	NON FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count	0	9	33	42
	% within age group	0%	21.4%	78.6%	100.0%
36-40 Yrs	Count	6	10	28	44
	% within age group	13.6%	22.7%	63.6%	100%
41-45 Yrs	Count	21	5	1	27
	% within age group	77.8%	18.5%	3.7%	100.0%
46-50 Yrs	Count	16	1	1	18
	% within age group	88.9%	5.6%	5.6%	100.0%
51-55 Yrs	Count	17	1	0	18
	% within age group	94.4%	5.6%	.0%	100.0%
56-60 Yrs	Count	5	1	0	6
	% within age group	83.3%	16.7%	.0%	100.0%
TOTAL		65	27	63	155
		41.9%	17.4%	40.6%	100.0%

Table: 3 Non fusion among males

AGE GROUP	MALES	OTHER GRADES	NON FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count	0	9	29	38
	% within age group	0%	23.7%	76.3%	100.0%
36-40 Yrs	Count	4	8	24	36
	% within age group	11.1%	22.2%	66.7%	100%
41-45 Yrs	Count	16	5	1	22
	% within age group	72.7%	22.7%	4.5%	100.0%
46-50 Yrs	Count	14	1	1	16
	% within age group	87.5%	6.2%	6.2%	100.0%
51-55 Yrs	Count	17	0	0	18
	% within age group	100.%	.0%	.0%	100.0%
56-60 Yrs	Count	4	0	0	4
	% within age group	100%	.0 %	.0%	100.0%
TOTAL		55	23	55	133
		41.4%	17.3%	41.4%	100.0%

36 – 40 years:

In the age group i.e. between 36 – 40 years, 10 cases (22.7 % within that age group) showed unilateral non fusion and 28 cases (63.6%) showed bilateral non fusion. Of the 10 cases of unilateral non fusion 8 cases were of males (Table: 3) and 2 cases belonged to females. Among 28 cases of bilateral non fusion 24 cases belonged to male and 4 cases belonged to female (Table: 4).

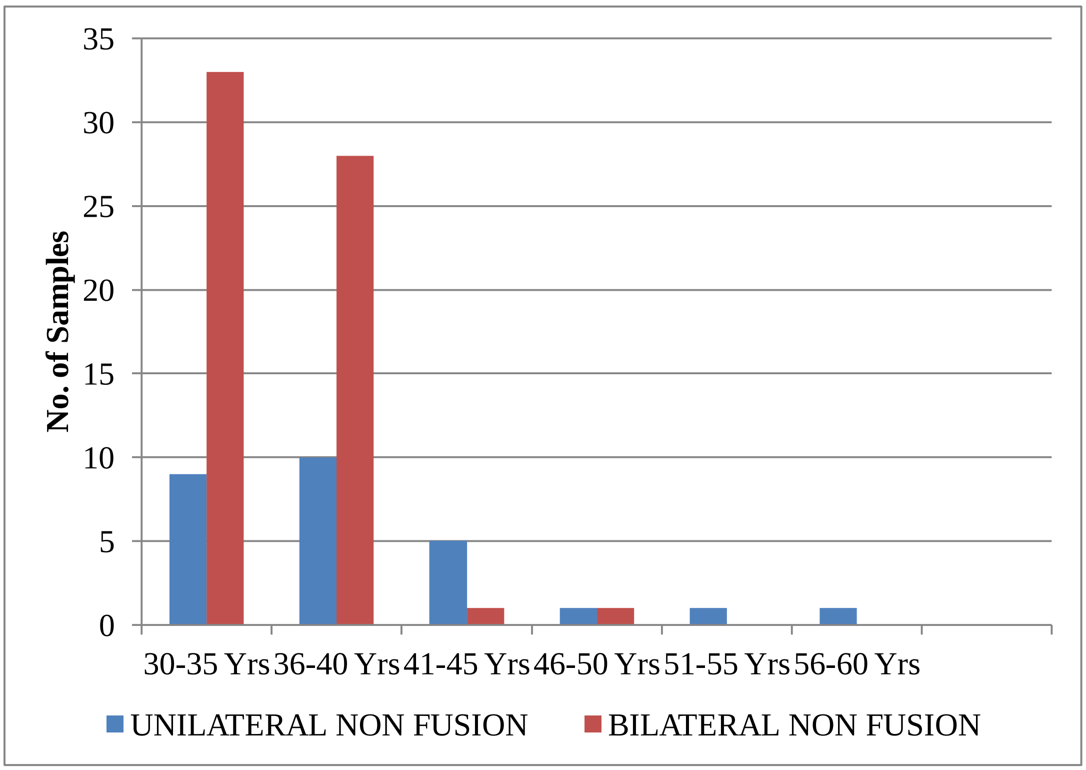


Fig – 3: Distribution of non fusion among the samples.

41 – 45 years: In this age group, 5 cases (18.5% within that age group) showed unilateral non fusion and 1case (3.7%) showed bilateral non fusion. All the 5 cases of unilateral non fusion were of males and the one case which showed bilateral non fusion was also male. None of the five female hyoid bones in this age group showed non fusion

Table: 4 Non fusion among females

AGE GROUP	FEMALES	OTHER GRADES	NON FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count % within age group	0 0%	0 0%	4 100%	4 100.0%
36-40 Yrs	Count % within age group	2 25.0%	2 25.0%	4 50.0%	8 100%
41-45 Yrs	Count % within age group	5 100%	0 .0%	0 .0%	5 100.0%
46-50 Yrs	Count % within age group	2 100%	0 .0%	0 .0%	2 100.0%
51-55 Yrs	Count % within age group	0 .0%	1 100.0%	0 .0%	1 100.0%
56-60 Yrs	Count % within age group	1 50.0%	1 50.0 %	0 .0%	2 100.0%
TOTAL		10 45.5%	4 18.2%	8 36.4%	22 100.0%

46 – 50 years:

In this class interval, 1 case (5.6%) showed unilateral non fusion which belonged to male and 1 case (5.6%) showed bilateral non fusion which also of male sex. None of the 2 female hyoid bones in this age group showed non fusion as in the previous class interval.

51 – 55 years:

As age increases the total number of cases which showed non fusion had been reduced (Fig 3). only one case showed unilateral non fusion (5.5 %) which as of female sex. Among 17 male cases none of one showed non fusion.

56 – 60 years:

In the last class interval, of the total 6 cases, only one (16.7%) female hyoid bone showed unilateral non fusion.

Commencement of fusion:

In the first class interval 30 – 35 years, 8 cases (19%) showed unilateral commencement of fusion, which belonged to male sex (Table: 5). 11 cases (25%)

showed unilateral commencement of fusion in the age group 36 – 40 years, out of which 8 were male and 3 were female cases.

Table: 5 Commencement of fusion among both sexes

AGE GROUP	BOTH SEXES	OTHER GRADES	COMMENCEMENT OF FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count % within age group	34 81%	8 19%	0 0%	42 100.0%
36-40 Yrs	Count % within age group	33 75%	11 25%	0 0%	44 100%
41-45 Yrs	Count % within age group	20 74.1%	7 25.9%	0 0%	27 100.0%
46-50 Yrs	Count % within age group	15 83.3%	3 16.7%	0 0%	18 100.0%
51-55 Yrs	Count % within age group	16 88.9%	2 11.1%	0 .0%	18 100.0%
56-60 Yrs	Count % within age group	5 83.3%	1 16.7 %	0 .0%	6 100.0%
TOTAL % within age group		123 79.4%	32 20.6%	0 .0%	155 100.0%

In the age group 41 – 45 years, totally 7 cases (25.9%) showed unilateral commencement of fusion, among which 5 were male and 3 were female. 3 cases (16.7%) showed unilateral commencement of fusion in the age group between 46 – 50 years and all the three were males (Table:6). In the age group 51 – 55 years, 2 cases (11.1%) showed unilateral commencement of fusion and each one of the case was a male and a female.

In the last class interval, only one hyoid bone (16.7%) showed unilateral commencement of fusion and that belonged to male sex.

Table: 6 Commencement of fusion - males

AGE GROUP	MALES	OTHER GRADES	COMMENCEMENT OF FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count	30	8	0	38
	% within age group	78.9%	21.1%	0%	100.0%
36-40 Yrs	Count	28	8	0	36
	% within age group	77.8%	22.2%	0%	100%
41-45 Yrs	Count	17	5	0	22
	% within age group	72.3%	22.7%	0%	100.0%
46-50 Yrs	Count	13	3	0	16
	% within age group	81.2%	18.8%	0%	100.0%
51-55 Yrs	Count	16	1	0	17
	% within age group	94.1%	5.9%	.0%	100.0%
56-60 Yrs	Count	3	1	0	4
	% within age group	75%	25 %	.0%	100.0%
TOTAL	Count	107	26	0	133
	% within age group	80.5%	19.5%	0%	100.0%

Partial fusion:

Partial fusion was not seen in any of the cases in the 30 – 35 age groups. 4 cases (9.1%) showed unilateral partial fusion and 1 case (2.3%) showed bilateral partial fusion in the next age group ie from 36 – 40 years. Out of 4 cases of unilateral partial fusion 3 cases were male and 1 was female. Bilateral partial fusion in the 1 case belonged to male sex (Table: 7).

In the class interval between 41 – 45 years, 5 cases (18.5%) showed unilateral partial fusion, which included 3 males and 2 females. Bilateral partial fusion was not seen in this age group. Two cases (11.1%) showed unilateral partial fusion, both belonged to male sex in the age group of 46 – 50 years .Female hyoid bones in this age group did not showed partial fusion. In the last two age groups i.e. from 51 – 55 years and 56 – 60 years, each group had one case which showed unilateral partial fusion and both of them were of male sex.

Table: 7 - Partial fusion both sexes

AGE GROUP	BOTH SEXES	OTHER GRADES	PARTIAL FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count	42	0	0	42
	% within age group	100%	0%	0%	100.0%
36-40 Yrs	Count	39	4	1	44
	% within age group	88.6%	9.1%	2.3%	100%
41-45 Yrs	Count	22	5	0	27
	% within age group	81.5%	18.5%	0%	100.0%
46-50 Yrs	Count	16	2	0	18
	% within age group	88.9%	11.1%	0%	100.0%
51-55 Yrs	Count	17	1	0	18
	% within age group	94.4%	5.6%	.0%	100.0%
56-60 Yrs	Count	5	1	0	6
	% within age group	83.3%	16.7 %	.0%	100.0%
TOTAL		141	13	1	155
		91.0%	8.4%	.6%	100.0%

Complete fusion:

In the first class interval between 30 – 35 years, only one case (2.4%) showed unilateral complete fusion which was of male hyoid bone. 5 cases (11.4%) in the age group 36 – 40 years showed unilateral complete fusion, out of which 3 were male and 2 were female

Table: 8 Complete fusion among both sexes.

AGE GROUP	BOTH SEXES	OTHER GRADES	COMPLETE FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count % within age group	41 97.6%	1 2.4%	0 0%	42 100.0%
36-40 Yrs	Count % within age group	39 88.6%	5 11.4%	0 .0%	44 100%
41-45 Yrs	Count % within age group	6 22.2%	7 25.9%	14 51.9%	27 100.0%
46-50 Yrs	Count % within age group	3 87.5%	2 6.2%	13 6.2%	18 100.0%
51-55 Yrs	Count % within age group	2 11.1%	1 5.6%	15 83.3%	18 100.0%
56-60 Yrs	Count % within age group	0 0%	2 33.3 %	4 66.7%	6 100.0%
TOTAL		91 41.4%	18 11.6%	46 29.7%	155 100.0%

7 cases (25.9%) showed unilateral complete fusion and 14 cases (51.9%) showed bilateral complete fusion in the age group of 41 – 45 years (Table: 8), (Fig 4) Among the 7 cases which showed unilateral complete fusion, 5 were male and 2 were female. Out of 14 cases which showed bilateral complete fusion, 12 were male and 2 belonged to female.

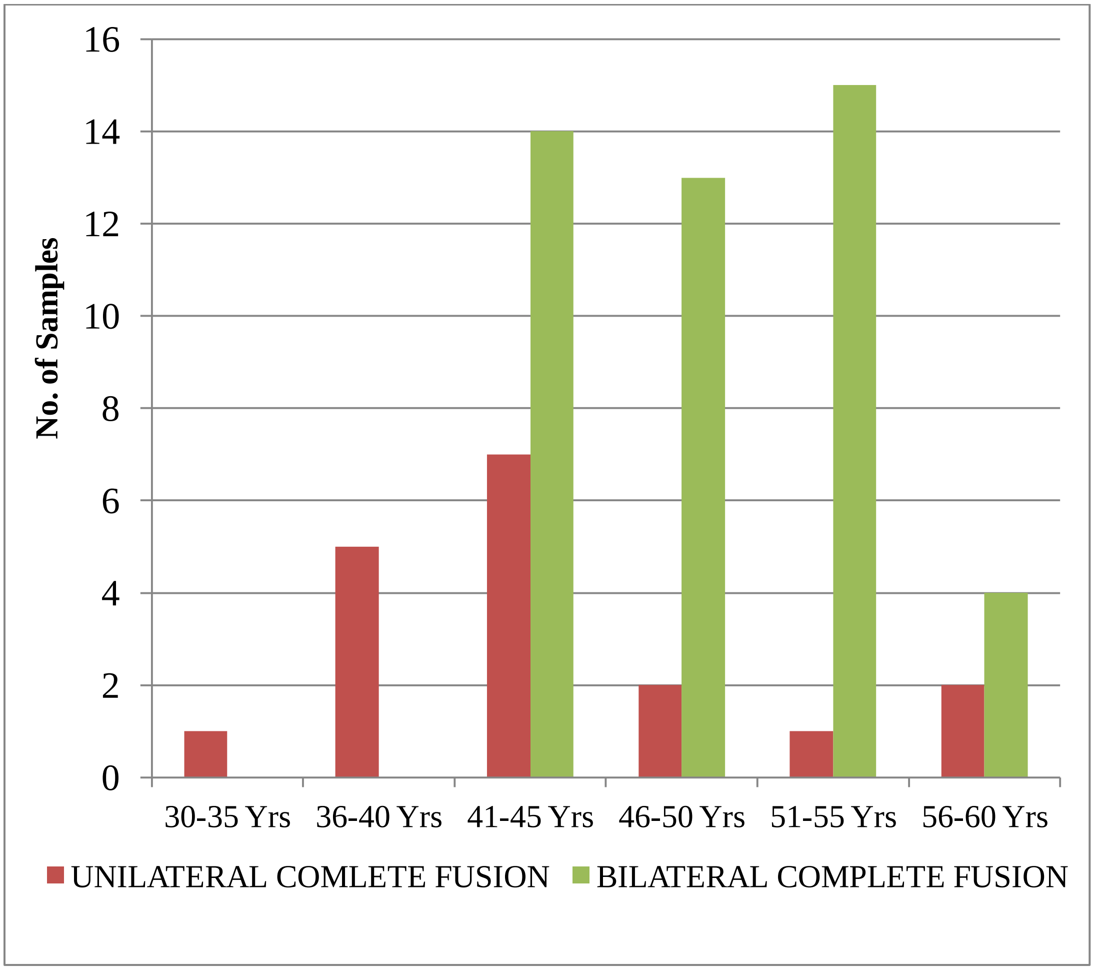


Fig 4: Distribution of unilateral and bilateral complete among the samples

In the age group between 46 – 50 years, 2 cases (11.1%) showed unilateral complete fusion and 13 cases (72.2%) showed bilateral complete fusion. Unilateral complete fusion which was seen in 2 cases belongs to male and 13 among bilateral complete fusion cases, 11 were male and 2 were female.

Table: 9 Complete fusion among males

AGE GROUP	MALES	OTHER GRADES	COMPLETE FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count % within age group	37 97.4%	1 2.6%	0 0%	38 100.0%
36-40 Yrs	Count % within age group	33 91.7%	3 8.3%	0 .0%	36 100%
41-45 Yrs	Count % within age group	5 22.7%	5 22.7%	12 54.5%	22 100.0%
46-50 Yrs	Count % within age group	3 18.8%	2 12.5%	11 68.8%	16 100.0%
51-55 Yrs	Count % within age group	1 5.9%	1 5.9%	15 88.2%	17 100.0%
56-60 Yrs	Count % within age group	0 0%	2 50 %	2 50%	4 100.0%
TOTAL		79 59.4%	14 10.5%	40 30.1%	133 100.0%

15 cases (83.3%) showed bilateral complete fusion and 1 (5.6%) case showed unilateral complete fusion in the fifth class interval i.e. between 51 – 55 years. All the cases which showed unilateral and bilateral complete fusion in this age group belonged to male (Table: 9).

In the last age group, between 55 – 60 years, 2 cases (33.3%) showed unilateral complete fusion, which belonged to male sex and 4 cases (66.7%) showed bilateral complete fusion. Out of the 4 cases of bilateral complete fusion, 2 cases were male and 2 cases were female (Table: 10).

Table: 10 Complete fusion – Females

AGE GROUP	FEMALES	OTHER GRADES	COMPLETE FUSION		TOTAL
			U/L	B/L	
30-35 Yrs	Count % within age group	4 100%	0 0%	0 0%	4 100.0%
36-40 Yrs	Count % within age group	6 75.0%	2 25.0%	0 .0%	8 100%
41-45 Yrs	Count % within age group	1 20%	2 40%	2 40%	5 100.0%
46-50 Yrs	Count % within age group	0 .0%	0 .0%	2 100%	2 100.0%
51-55 Yrs	Count % within age group	1 100%	0 .0%	0 .0%	1 100.0%
56-60 Yrs	Count % within age group	0 .0%	0 .0 %	2 100%	2 100.0%
TOTAL		12 54.5%	4 18.2%	6 27.3%	22 100.0%

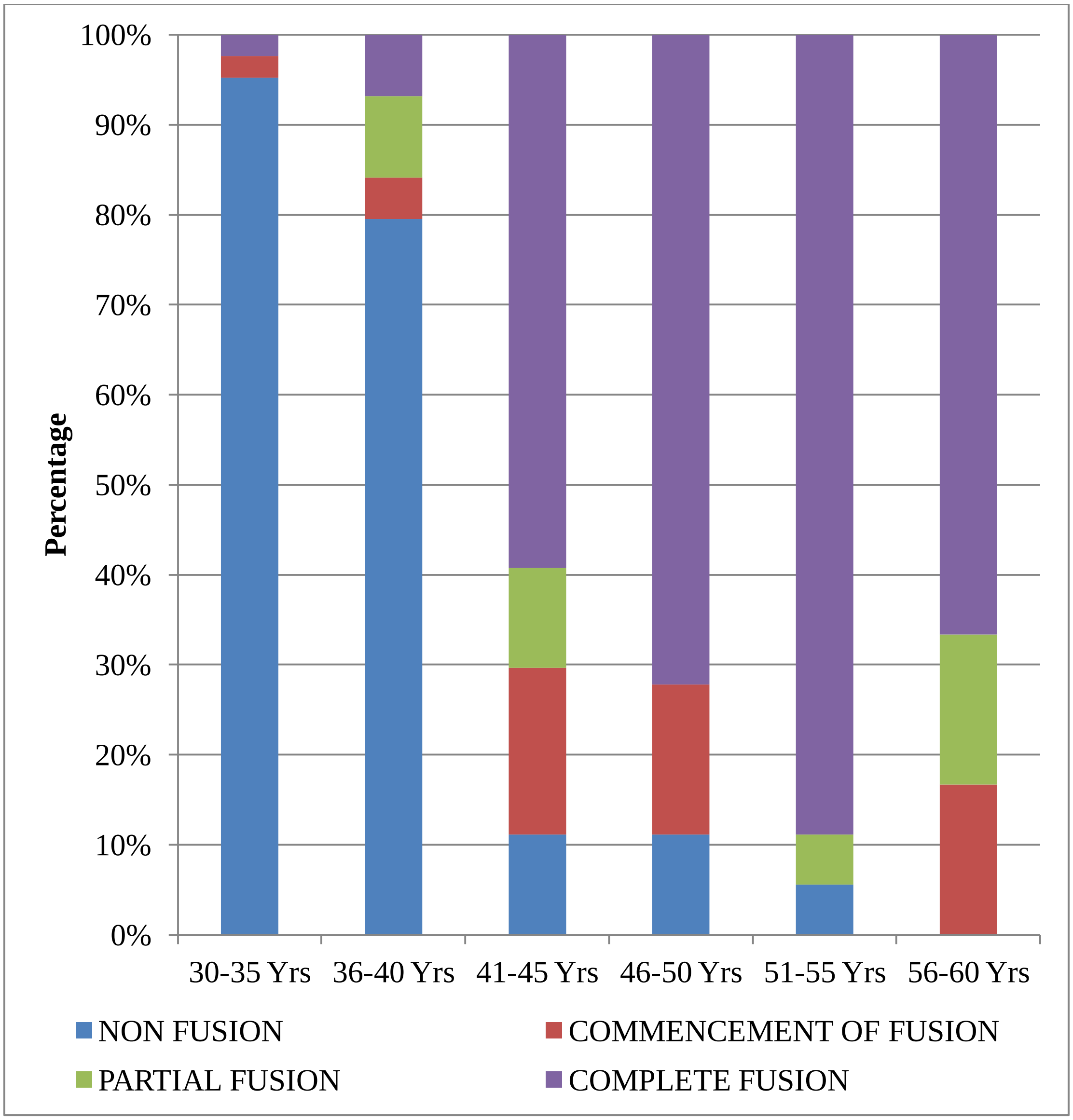


Fig 5: Distribution of radiological findings among the samples

Table: 11 Unilateral fusion of hyoid bone in males and females

SIDE OF FUSION	MALES	FEMALES
Left side	11(73.33%)	1 (25%)
Right side	4 (26.66%)	3 (75%)
Total	15 (100%)	4 (100%)

Histological grading:

Out of 42 hyoid bones in the age group of 30-35 years; 30(71.4%) cases were in grade I, 11(26.2%) cases in grade II and 1(2.4%) case in grade III. In the age group of 36-40 years , 44 hyoid bones were present , out of which 13(29.5%) cases were in grade-I; 31 (70.5%) cases in grade-II. In the age group of 41-45 years, 28 hyoid bones were present, among which 15 (53.6%) cases in grade-II and 13 (46.4%) cases in grade- III (Fig. 6).

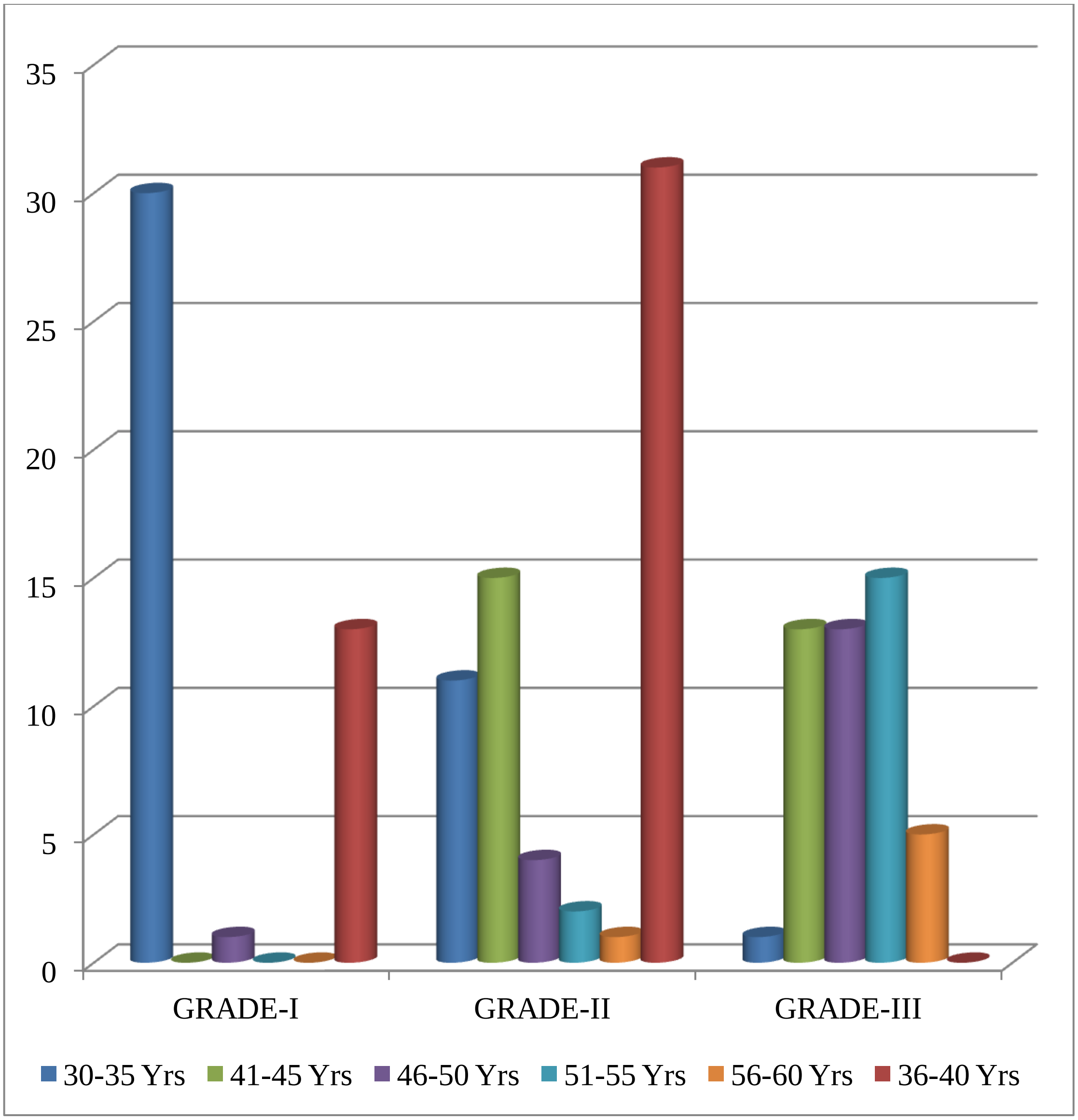


Fig: 6 Distribution of samples in different grades of ossification based on histology.

Table: 12 Histological grading among both sexes

AGE GROUP	BOTH SEXES	HISTOLOGY GRADING			TOTAL
		GRADE-I	GRADE-II	GRADE-III	
30-35 Yrs	Count	30	11	1	42
	% within age group	71.4%	26.2%	2.4%	100%
36-40 Yrs	Count	13	31	0	44
	% within age group	29.5%	70.5%	0%	100%
41-45 Yrs	Count	0	15	13	28
	% within age group	0%	53.6%	46.4%	100%
46-50 Yrs	Count	1	4	13	18
	% within age group	5.6%	22.2%	72.2%	100%
51-55 Yrs	Count	0	2	15	17
	% within age group	0%	11.8%	88.2%	100%
56-60 Yrs	Count	0	1	5	6
	% within age group	0%	7.7%	83.3%	100%
TOTAL	Count	44	64	47	155
	% within age	28.4%	41.3%	30.3%	100%

Out of 18 hyoid bones present in the age group 46-50 years, 1 (5.6%) case showed grade I, 4(22.2%) and 13(72.2%) cases were present in grade II and grade III respectively (Table:12). Out of 17 hyoid bones in the age group 51- 55years, 2 cases represent grade II and 15 cases (82.2%) showed grade III. In the last age group of 56-60 years, 5 (83.3%) cases were in grade III and 1(16.7%) case was in the grade II(Fig 5)

DISCUSSION

The joint connecting the body and greater horn of the hyoid bone is initially cartilaginous, but after the middle age they became ossified, although there exists marked individual variations. In the present study, an attempt is made to classify fusion between the body and greater horn into various categories as according to the study done by Kaur Harjeet[11] et al. This will enable to narrow down the age of ossification of hyoid bone.

Parsons[26] stated in his study that bilateral fusion may occur in 30 – 40 years and is quite rare until fifth decade of life. Vij[23] conducted studies in Indian population and arrived the conclusion that fusion between the body and greater horn would occur during 40 – 60 years of age, while Mukherjee[24] stated that it was between 40 – 50 years.

Vij had cited a study of 110 excised hyoid bones which had complete fusion of the greater horn with the body in 39 subjects and partial fusion in 14 subjects. The youngest age in whom fusion occurred was 18 years on the other hand there were individuals in the eighth and ninth decades where no fusion was found at all. In the present study, among the total number of cases, 46 cases showed complete fusion on both sides and 14 cases showed partial fusion.

In the age group between 30 – 60 years, the youngest age in whom bilateral complete fusion occurred is 42 years of which 2 cases are male and only one case is female. Proportion of cases with bilateral fusion is highest in the age group between 51 – 55 years with 83.3 % of cases having bilateral fusion in males in this age group. Females showed 100% bilateral fusion in the age group between 55 – 60 years whereas males showed only 50% bilateral fusion in that age group.

As stated by Deepak H D Souza et al15, as age advances, there was a decrease in bilateral non fusion and increase in unilateral and bilateral fusion15. This is also proved in this study. 78.6 % of cases showed bilateral non fusion in the age group of 31 – 35 years whereas none of the case showed either unilateral or bilateral complete fusion in that age group.

The mean age of bilateral fusion was 42 years in the study conducted by Deepak H D Souza et al from the cases aged between 12 – 70 years. In this study, the mean age of bilateral complete fusion is 50 years (Table 13) This is much higher than the mean age as stated in the study by Deepak H D Souza et al. The reason for this difference in mean age of bilateral complete fusion is that majority of the cases were aged beyond 40 years in the present study and majority of cases aged less than 40 years in the previous study by Deepak H D Souza et al[15].

In the present study, one case showed unilateral fusion at the age of 57 years. This proved that unilateral fusion may not be followed by fusion on the other side. This observation coincides with earlier studies. Different parts of the skeleton can thus age at different rates both within and between the individuals.

Both Miller and Parson found that many elderly individuals had either unilateral or bilateral non fusion. This is also proved positive in this study. Bilateral non fusion is found at the age of 50 years and unilateral non fusion is found at the age of 55 years and both belonged to male sex.

Miller[20] in his study did not found any significant sex difference between men and women in proportion of individuals with bilateral fusion . In this study the mean age of bilateral fusion in male is 50 years and in female it is 49 years. As stated in previous study, statistically no significant difference is observed between male and female. Hence, fusion of the body and greater cornu of the hyoid bone cannot be regarded as a sex indicator.

Table: 13 Mean age ± SD of fusion in the hyoid bone

DEGREE OF FUSION	MALE	FEMALE	BOTH SEXES	p VALUE
Bilateral non fusion	37.126 ±4.5	38.16± 5.9	37.26± 4.7	0.480

Unilateral fusion	45.64±7.2	42± 3.5	44.83± 6.6	0.350
Bilateral fusion	49.77± 4.7	48.83± 6.5	49.65±4.9	0.669

In O' Halloran[16], Lundy et al study, females showed high frequency of unilateral non fusion. In the present study, unilateral non fusion is seen in 17.3 % of males and 18.2% of females. Thus females showed relatively higher degree of non fusion than males in all the age groups.

Parsons in his study showed that there is no specific pattern to show that one side of the hyoid bone fuses earlier than the other. In this study, out of 155 hyoid bones, 11 hyoid bones are fused on right side and 7 hyoid bones are fused on left side. This showed that right side fused earlier than the left. This finding contrast to the findings of Parsons and Atul Gupta et al[12].

The present study agreed with the previous study. In this study, 71.4% of cases are in grade I and 2.4% of cases are in grade III in the age group between 30 – 35 years. At 55 – 60 years, 83.3% of cases are in grade III , 16.7% of the cases showed grade II and none of the case in this age group is in grade I. In the study by Hiroyasu Kanetaka et al based on histological grading of the joint between body and greater cornu of the hyoid bone, it was stated that in young adult 75% was in grade I and 14.1% was in grade III whereas in elderly population 26% of cases were in grade I and 35.9% of the cases were in grade III. As age advances, the degree of fusion towards grade III increases.

The following steps could have been taken to improve the results of this study:

1. Sample selection: The age group of the samples from the autopsy cases could have been taken both in the younger and elder age groups i.e. less than 30 years and more than 60 years so that better analysis of the study samples might be done.
2. The breadth of the joint space between the body and the greater cornu of the hyoid bone could have been measured by using standardized scale.
3. In histological examination, serial sections of the junction between the body and greater horn of the hyoid bone could have been taken for better analysis of different grades of ossification(as done by Kanetaka et al in their study based on the histological grading of the joint between body and greater cornu of the hyoid bone)

CONCLUSION

The incidence of fusion of the body and the greater cornu of the hyoid bone increases with age and maximum number of hyoid bones with bilateral complete fusion are observed in the age group of 51 – 55 years. Both in radiological and histological analysis, increased frequency of complete fusion are observed in the age group of 51 – 55 years. In the present study, the age group of complete fusion is higher than the previous studies, the reason for this difference is that majority of the cases included in this study are aged beyond 40 years. Age estimation from teeth, skull and pelvis has been done conventionally but each method has its own limitations and they are governed by endocrine, racial, dietary and sex factors. These methods can be used to categorize age into broad age groups and estimation of age based on hyoid bone can be used as a supplement to the above mentioned methods.

As both radiological and histological findings coincides with the age of fusion of hyoid bone, estimating the age group based on ossification of the body and greater horn of the hyoid bone can be done alone with radiological study. As histological study of the ossification of the hyoid bone, is more time consuming, it is concluded that radiological assessment of the hyoid bone alone is sufficient in determining the age of an individual. In this study, it is observed that, bilateral non fusion is present in the samples of age less than 40 years and bilateral completion fusion is present in samples of age above 50 years. No sex difference is observed in the age of bilateral fusion of the hyoid bone. Factors affecting the ossification process should be noted before estimating the age of an individual based on radiological examination of the bone.

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
	7	2.1.12	57	1.1	Complete fusion	Partial fusion	Right	GRADE-
	13	2.1.12	45	1.2	Partial fusion	Complete fusion	Right	GRADE-
	59	7.1.12	34	1.3	Non fusion	Non fusion	Right	GRADE
	66	8.1.12	36	1.4	Non fusion	Non fusion	Right	GRADE-
	129	16.1.12	54	1.5	Complete fusion	Complete fusion	Right	GRADE-
	208	24.1.12	38	1.6	Commencement of fusion	Non fusion	Right	GRADE-
	347	11.2.12	38	1.7	Commencement of fusion	Non fusion	Right	GRADE-
	372	15.2.12	42	1.8	Complete fusion	Complete fusion	Left	GRADE-
	401	18.2.12	36	1.9	Commencement of fusion	Non fusion	Right	GRADE-
	413	20.2.12	51	1.1	Complete fusion	Complete fusion	Right	GRADE-
	417	21.2.12	57	1.11	Complete fusion	Complete fusion	Right	GRADE-
	440	23.2.12	36	1.12	Non fusion	Non fusion	Right	GRADE
	465	26.2.12	39	1.13	Complete fusion	Partial fusion	Left	GRADE-
	468	27.2.12	45	1.14	Complete fusion		Right	GRADE-
			42	1.15	Commencement of fusion	Partial fusion	Right	GRADE-
	499	1.3.12	32	1.16	Non fusion	Non fusion	Right	GRADE
	527	3.3.12	40	2.1	Commencement of fusion	Non fusion	Left	GRADE
	532	3.3.12	45	2.2	Complete fusion	Complete fusion	Right	GRADE-
	543	5.3.12	45	2.3	Complete fusion	Complete fusion	Right	GRADE-
	555	7.3.12	35	2.4	Non fusion	Non fusion	Left	GRADE
	562	7.3.12	55	2.5	Complete fusion	Complete fusion	Right	GRADE-
	583	10.3.12	45	2.6	Complete fusion	Complete fusion	Right	GRADE-
	630	16.3.12	55	2.7	Complete fusion	Complete fusion	Right	GRADE-
	647	18.3.12	38	2.8	Non fusion	Non fusion	Left	GRADE-
	710	27.3.12	38	2.9	Non fusion	Non fusion	Left	GRADE
	791	7.4.12	42	2.1	Complete fusion	Commencement of fusion	Left	GRADE-
	803	9.4.12	39	2.11	Non fusion	Non fusion	Right	GRADE-
	871	17.4.12	35	2.12	Non fusion	Non fusion	Right	GRADE
	897	19.4.12	47	2.13	Complete fusion	Commencement of	Left	GRADE-

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
						fusion		
	941	22.4.12	45	2.14	Complete fusion	Commencement of fusion	Left	GRADE-
	947	23.4.12	55	2.15	Complete fusion	Complete fusion	Right	GRADE-
	984	26.4.12	40	2.16	Non fusion	Non fusion	Right	GRADE-
	999	28.4.12	33	3.1	Commencement of fusion	Non fusion	Left	GRADE
	1110	28.4.12	45	3.2	Complete fusion	Complete fusion	Right	GRADE-
	1021	30.4.12	35	3.3	Commencement of fusion	Non fusion	Right	GRADE-
	1023	30.4.12	33	3.4	Non fusion	Non fusion	Right	GRADE
	1038	2.5.12	52	3.5	Complete fusion	Complete fusion	Left	GRADE-
	1039	2.5.12	46	3.6	Complete fusion	Complete fusion	Left	GRADE-
	1044	3.5.12	35	3.7	Non fusion	Non fusion	Left	GRADE-
	1065	4.5.12	38	3.8	Non fusion	Non fusion	Left	GRADE
	1069	4.5.12	35	3.9	Non fusion	Non fusion	Left	GRADE
	1114	9.5.12	34	3.1	Non fusion	Non fusion	Left	GRADE-
	1091	6.5.12	45	3.11	Complete fusion	Complete fusion	Left	GRADE-
	1097	7.5.12	40	3.12	Commencement of fusion	Complete fusion	Right	GRADE-
	1158	14.5.12	35	3.13	Non fusion	Non fusion	Right	**GRADE**
	1167	15.5.12	38	~~3.14~~	Complete fusion	Partial fusion	Right	GRADE-
	1209	19.5.12	39	3.15	Non fusion	Non fusion	Right	GRADE-
	1211	19.5.12	36	3.16	Non fusion	Non fusion	Right	GRADE
	1219	20.5.12	35	4.1	Commencement of fusion	Non fusion	Left	GRADE-
	1214	20.5.12	40	4.2	Partial fusion	Complete fusion	Right	GRADE-
	1219	20.5.12	35	4.3	Non fusion	Non fusion	Right	GRADE
	1223	21.5.12	47	4.4	Complete fusion	Complete fusion	Right	GRADE-
	1234	21.5.12	40	4.5	Non fusion	Non fusion	Right	GRADE-
	1241	22.5.12	58	4.6	Complete fusion	Complete fusion	Left	GRADE-
	1245	23.5.12	55	4.7	Complete fusion	Complete fusion	Left	GRADE-
	1281	25.5.12	39	4.8	Non fusion	Non fusion	Right	GRADE-
	1288	26.5.12	34	4.9	Non fusion	Non fusion	Right	GRADE
	1289	26.5.12	55	4.11	Complete fusion	Complete fusion	Right	GRADE-1

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
	1324	29.5.12	30	4.12	Non fusion	Non fusion	Right	GRADE-
	1728	10.7.12	39	4.13	Non fusion	Non fusion	Right	GRADE-
	1331	29.5.12	40	4.14	Complete fusion	Commencement of fusion	Left	GRADE-
	1343	30.5.12	35	4.15	Commencement of fusion	Non fusion	Left	GRADE
	1345	31.5.12	38	4.16	Commencement of fusion	Non fusion	Left	GRADE-
	1357	1.6.12	35	5.1	Commencement of fusion	Non fusion	Right	GRADE-
	1399	4.6.12	44	5.2	Complete fusion	Complete fusion	Right	GRADE-
	1404	5.6.12	33	5.3	Non fusion	Non fusion	Right	GRADE
	1417	7.6.12	38	5.4	Non fusion	Non fusion	Right	GRADE-
	1424	8.6.12	38	5.5	Non fusion	Non fusion	Right	GRADE-
	1442	10.6.12	42	5.6	Complete fusion	Complete fusion	Right	GRADE-
	1456	11.6.12	39	5.7	Non fusion	Non fusion	Right	GRADE-
	1492	15.6.12	42	5.8	Complete fusion	Complete fusion	Right	GRADE-
	1500	16.6.12	35	5.9	Non fusion	Non fusion	Right	GRADE
	1504	16.6.12	34	5.1	Non fusion	Non fusion	Right	GRADE
	1515	17.6.12	60	5.11	Complete fusion	Complete fusion	Right	GRADE-
	1517	18.6.12	37	5.12	Non fusion	Non fusion	Right	GRADE-
	1530	19.6.12	38	5.13	Non fusion	Non fusion	Right	GRADE-
	1543	20.6.12	42	5.14	Complete fusion	Commencement of fusion	Left	GRADE-
	1550	21.6.12	56	5.15	Complete fusion	Complete fusion	Right	GRADE-
	1564	23.6.12	44	5.16	Complete fusion	Complete fusion	Right	GRADE-
	1573	1736	55	6.1	Commencement of fusion	Non fusion	Right	GRADE-
	1525	18.6.12	32	6.2	Non fusion	Non fusion	Right	GRADE
	1580	25.6.12	55	6.3	Non fusion	Complete fusion	Left	GRADE-
	1582	25.6.12	55	6.4	Complete fusion	Complete fusion	Left	GRADE-
	1593	26.6.12	35	6.5	Non fusion	Commencement of fusion	Right	GRADE-
	1616	28.6.12	52	6.6	Complete fusion	Complete fusion	Left	GRADE-
	1621	29.6.12	54	6.7	Complete fusion	Complete fusion	Right	GRADE-

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
	1626	29.6.12	55	6.8	Complete fusion	Complete fusion	Right	GRADE-
	1630	29.6.12	35	6.9	Non fusion	Non fusion	Right	GRADE-
	1655	1.7.12	47	6.1	Complete fusion	Commencement of fusion	Left	GRADE-
	1683	5.7.12	35	6.11	Non fusion	Non fusion	Right	GRADE-
	1705	7.7.12	36	6.12	Non fusion	Non fusion	Right	GRADE
	1745	11.7.12	40	6.13	non fusion	Non fusion	Right	GRADE-
	1669	4.7.12	47	6.14	Complete fusion	Complete fusion	Right	GRADE-
	1548	21.6.12	33	6.15	Non fusion	Non fusion	Right	GRADE
	1559	23.6.12	35	6.16	Commencement of fusion	Non fusion	Left	GRADE-
	1575	24.6.12	48	7.1	Partial fusion	Non fusion	Left	GRADE-
	1683	5.7.12	35	7.2	Non fusion	Non fusion	Right	GRADE
	1691	6.7.12	37	7.3	Non fusion	Non fusion	Right	GRADE
	1699	4.7.12	47	7.4	Complete fusion	Complete fusion	Left	GRADE-
0	1667	3.7.12	45	7.5	Complete fusion	Complete fusion	Left	GRADE-
1	1717	8.7.12	35	7.6	Commencement of fusion	Non fusion	Left	GRADE
2	1805	18.7.12	34	7.7	Non fusion	Non fusion	Left	GRADE
3	1806	18.7.12	48	7.8	Partial fusion	Commencement of fusion	Left	GRADE-
4	1813	19.7,12	33	7.9	Non fusion	Non fusion	Right	GRADE
5	1831	2O.7.12	44	7.1	Complete fusion	Complete fusion	Right	GRADE-
6	1834	20.7.12	55	7.11	Complete fusion	Complete fusion	Right	GRADE-
7	1836	21.7.12	30	7.12	Non fusion	Non fusion	Right	GRADE
8	1848	23.7.12	60	7.13	Complete fusion	Commencement of fusion	Left	GRADE-
9	1852	23.7.12	49	7.14	Complete fusion	Complete fusion	Right	GRADE-
0	1828	20.7.12	36	7.15	Non fusion	Non fusion	Left	GRADE-
1	1817	19.7.12	35	8.1	Non fusion	Non fusion	Right	GRADE
2	1856	23.7.12	47	8.2	Complete fusion	Complete fusion	Right	GRADE-
3	1847	22.7.12	33	8.3	Non fusion	Non fusion	Left	GRADE
4	1869	25.7.12	45	8.4	Complete fusion	Non fusion	Left	GRADE-
5	1859	24.7.12	50	8.5	Complete fusion	Complete fusion	Right	GRADE-
6	1862	24.7.12	38	8.6	Non fusion	Non fusion	Right	GRADE-

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
7	1869	25.7.12	45	8.7	Complete fusion	Complete fusion	Right	GRADE-
8	1871	25.7.12	35	8.8	Non fusion	Non fusion	Right	GRADE
9	1877	26.7.12	48	8.9	Complete fusion	Complete fusion	Left	GRADE-
0	1885	27.7.12	37	8.1	Non fusion	Non fusion	Right	GRADE
1	1886	27.7.12	37	8.11	Non fusion	Non fusion	Right	GRADE
2	1910	2.8.12	38	8.12	Partial fusion	Partial fusion	Right	GRADE-
3	1904	30.7.12	33	8.13	Non fusion	Non fusion	Right	GRADE
4	1906	31.7.12	32	8.14	Non fusion	Non fusion	Right	GRADE-
5	1909	1.8.12	45	8.15	Complete fusion	Complete fusion	Right	GRADE-
6	1910	2.8.12	38	9.1	Non fusion	Non fusion	Right	GRADE-
7	1922	3.8.12	32	9.2	Non fusion	Non fusion	Left	GRADE
8	1923	3.8.12	50	9.3	Complete fusion	Complete fusion	Left	GRADE-
9	1942	4.8.12	45	9.4	Non fusion	Partial fusion	Right	GRADE-
0	1925	3.8.12	55	9.5	Complete fusion	Complete fusion	Right	GRADE-
1	1952	5.8.12	32	9.6	Non fusion	Non fusion	Right	GRADE-
2	1950	4.8.12	45	9.7	Partial fusion	Non fusion	Left	GRADE-
3	1953	5.8.12	36	9.8	Non fusion	Non fusion	Left	GRADE
4	1962	6.8.12	37	9.9	Commencement of fusion	Non fusion	Left	GRADE-
5	1968	6.8.12	49	9.1	Complete fusion	Complete fusion	Right	GRADE-
6	1979	8.8.12	31	9.11	Non fusion	Non fusion	Left	GRADE
7	1978	8.8.12	46	9.12	Complete fusion	Complete fusion	Left	GRADE-
8	1982	9.8.12	42	9.13	Non fusion	Commencement of fusion	Right	GRADE-
9	1989	9.8.12	45	9.14	Non fusion	Partial fusion	Right	GRADE-
0	2006	11.8.12	35	9.15	Non fusion	Non fusion	Right	GRADE
1	2008	11.8.12	54	10.1	Complete fusion	Complete fusion	Left	GRADE-
2	2019	13.8.12	39	10.2	Non fusion	Commencement of fusion	Right	GRADE-
3	2025	13.8.12	40	10.3	Non fusion	Partial fusion	Right	GRADE-
4	2035	15.8.12	40	10.4	Commencement of fusion	Non fusion	Left	GRADE-
5	2029	14.8.12	49	10.5	Complete fusion	Complete fusion	Right	GRADE-
6	2028	14.8.12	32	10.6	Non fusion	Non fusion	Right	GRADE
7	2038	15.8.12	40	10.7	Commencement of	Non fusion	Left	GRADE-

O	P.M NUMBER	DATE	AGE	X-RAY NUMBER	RADIOLOGICAL FINDINGS		HISTOLOGY Right/ Left	HISTOLO GRADIN
					RIGHT	LEFT		
					fusion			
8	2042	16.8.12	48	10.8	Complete fusion	Complete fusion	Right	GRADE-
9	2047	17.8.12	55	10.9	Commencement of fusion	Partial fusion	Right	GRADE-
0	2045	16.8.12	35	10.1	Non fusion	Complete fusion	Right	GRADE
1	2074	19.8.12	40	10.11	Non fusion	Non fusion	Right	GRADE
2	2085	20.8.12	50	10.12	Non fusion	Non fusion	Right	GRADE
3	2057	17.8.12	45	10.13	Complete fusion	Complete fusion	Left	GRADE-
4	2093	21.8.12	45	10.14	Non fusion	Non fusion	Right	GRADE-
5	2025	12.8.12	40	10.15	Non fusion	Non fusion	Right	GRADE

Photoradiograph showing bilateral complete fusion of the hyoid bone

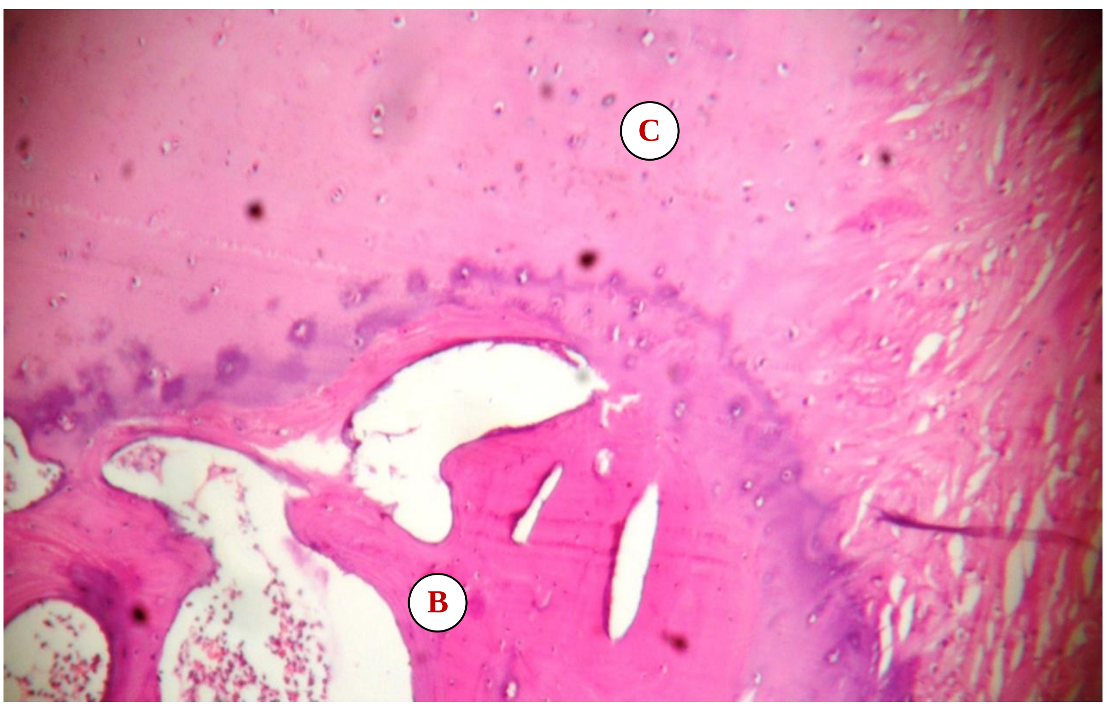
Photoradiograph showing bilateral non fusion of the hyoid bone

Photoradiograph showing commencement of fusion on the right side and non fusion on the left side of the hyoid bone

Photoradiograph showing partial fusion on the right side and non fusion on the left side of the hyoid bone.

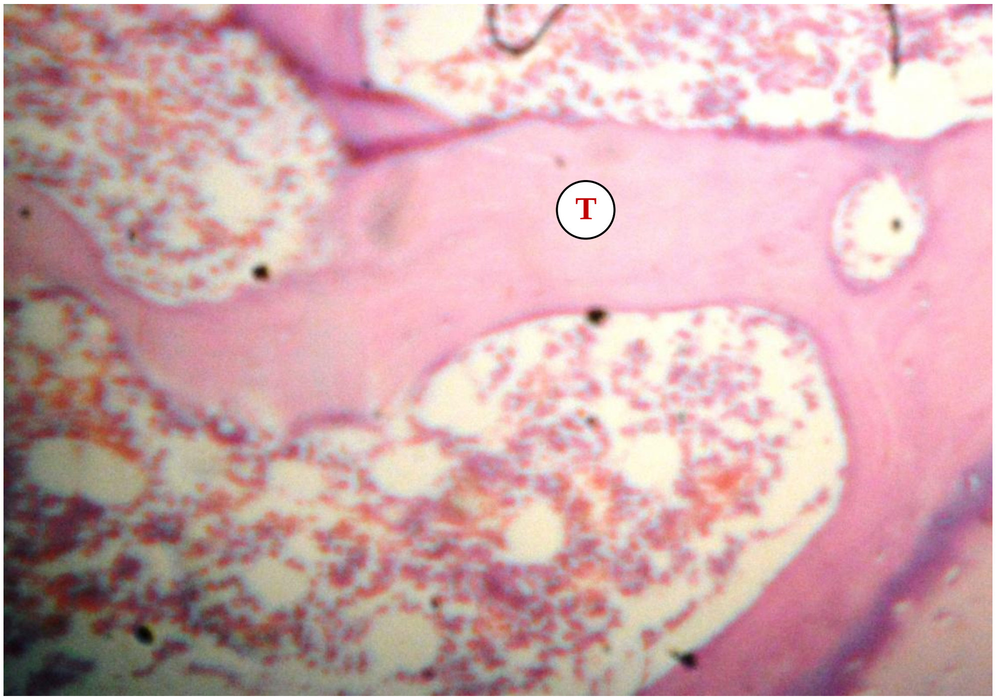
Hyoid bone along with numbered token.

Bit from the junction of body and greater cornu of the hyoid bone.



Photomicrograph showing fibrocartilage (C) and matured bone (B) – Grade-I

Photomicrograph showing specks of calcification between the matured bone (B) and fibrocartilage (C) – Grade-II



Photomicrograph showing mature bone with trabaculae formation (T) - Grade III.

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