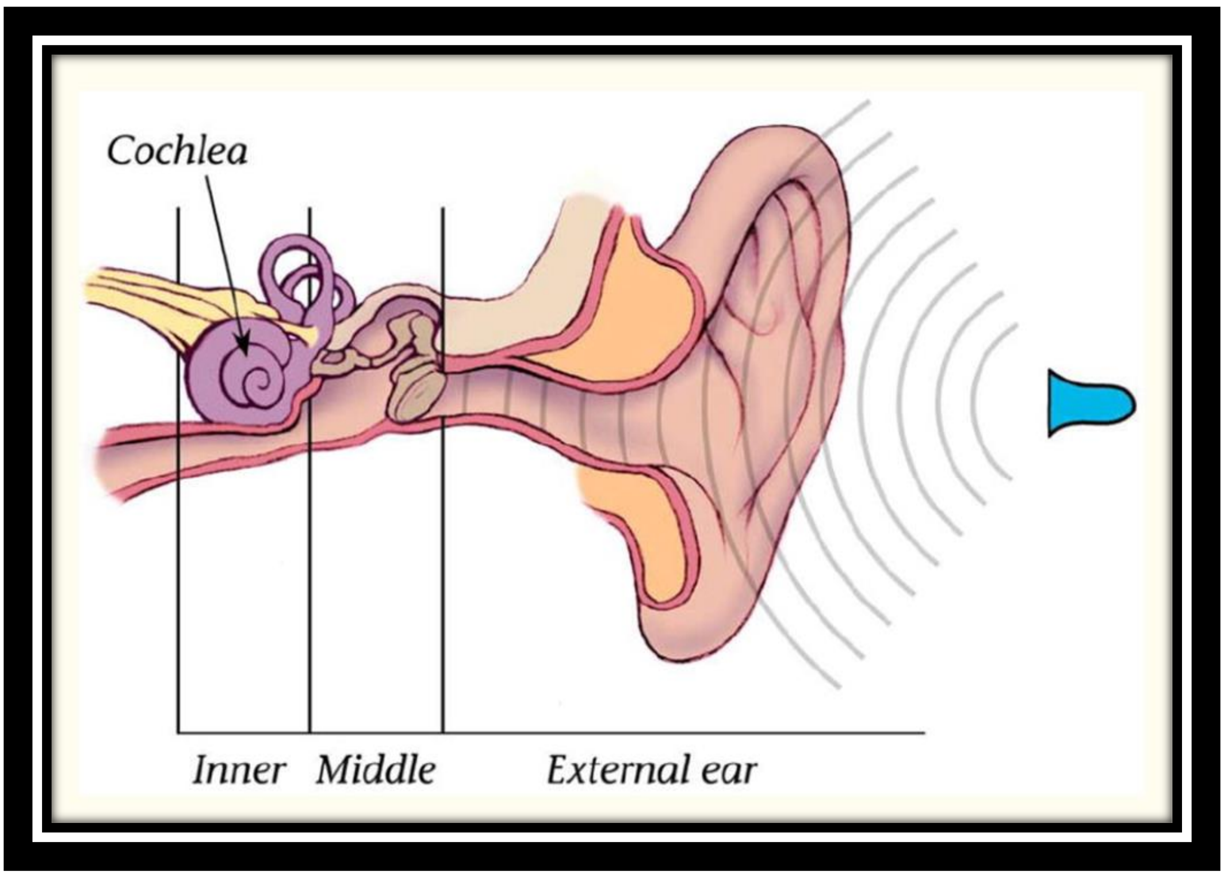
CHAPTER 1

INTRODUCTION

1.1 General context of the study

Worldwide, hearing is considered to be an essential sense because it influences the mental, physical and social well-being of an individual. Hearing is one of the dominant senses and similar to vision also comprehend for faraway cautioning and communication. It is used to alarm, deliver comfort and horror (Sundar, Chowdhury & Kamarthi, 2021). It is an informed recognized vibration anticipated as sound. To do so, a suitable signal should arrive at the advanced portions of the brain. The ability of the ear is to modify over actual vibration into a programmed apprehensive drive, this tends to be thought as a natural earpiece. Like a headset the ear is animated by vibration: in the earpiece the vibration changes over into an electrical signal and then into an anxious motion in the ear in the form of sound which thusly is then handled by the focal audible conduits of the mind. This kind of mechanism is very complex to accomplish (Priyadharshini, Arivazhagan & Arun, 2021).

Ear, the paired organ that is present separately on each side of the head with the actual receptor named as cochlea. Ear gives two significant function that is of hearing and equilibrium which generally relies upon particular receptors that are hair cells. Ear is a sensitive structure internally which needs to be dealt with great care in order to prevent any serious consequences. The conduction of sound inside the ear primarily begins when sound waves enter the canal and vibrate the eardrum (Figure 1.1) (Kuru et al., 2016). Ear ossicles, miniature bones “malleus, incus and stapes” transfer these vibrations to oval window (a sheath accessing to inner ear) (Davis & Silverman, 1970).



Pressure waves from the speaker pass through the air to the external ear which collects the sound and passes it to the ear drum. The middle ear consists of the ear drum, the middle ear bones, and the membrane over the oval window at the entrance to the inner ear. The cochlea of the inner ear is named with the Greek word for "snail"

Figure: 1.1 Sound transmission (Brownell, 1997)

1.1.1 Background

In human beings, referring to the auditory ossicles, some scientists concluded that it reaches to an absolute dimension and form in fetal periods and postnatal alterations are negligible, whereas there are certain studies which oppose this claim. These claims provoked us to make an attempt to discover out the developmental pattern of the ossicles and linking the facts with adults. Similar findings were reported that auditory ossicles reach their adult size in fetus by 6 months of intrauterine life (IUL) (Nadeem, 2012). Then some studies also documented the presence of adult sized ossicles in human fetuses of 5th month IUL and that their development is not fixed to rate of body growth in general. Conversely, there are studies which oppose this claim. A study reported that the development of auditory ossicles in humans is not completed during fetal life. The different parameters in the fetal periods increased adequately in the post-natal life (Rolvien et al., 2018).

Mostly many ear related disorders are self-inflicted that are caused during trauma, washing, ablution and inserting an object into the ear canal for the purpose of cleaning which interferes with the natural cleaning mechanism of an individual’s ear (Abu-Naser & Abu Hasanein, 2016). Ear ossicles which are a part of ossicular chain are important for proper sound conduction in ear. It is a great deal for an ENT specialist to completely restore the functional integrity of this structure, so in order to do it they need to have complete knowledge about the anatomy of ear ossicles (Entsfellner et al., 2015).

There are articles that mention the morphological variations in these structures but the information available is meagre to record these changes as all are cadaveric studies. In audition ear ossicles are critical structures and once in a while safeguarded in fossils (Kist & Scheifele, 2019).

Normally, the sound pressure difference that is created between the middle and external ear is in charge of transmission of sound that leads to vibrations in the ossicles with the assistance of tympanic membrane (Dawood, 2017).

Ear is isolated into three sections outside, center and internal ear. The ear ossicles incorporate three little bones that are malleus, incus and stapes. In tympanic cavity it frames a connection between the oval window medially and with tympanic membrane laterally (Quam, Coleman & Martinez, 2014).

Most laterally in the cavity is a presence of hammer like bone called malleus. It has 2 processes (anterior and lateral) a head, neck and handle also known as manubrium. Incus appears as anvil and has 2 processes (short and long) and a body. In incus at long process's tip, a small mass is present known as lenticular process; the head of stapes articulates with it. Stapes is the littlest of all the bones in the body that seems like a little stirrup and comprises of a little head on top, two crura, a base also called footplate and a neck (Saha, Srimani & Mazumdar, 2017). Malleus articulating surface is in contact with incus body by a synovial joint. Stapes head articulates with the lenticular process and forms an 'incudostapedial' joint (Mason, 2016). The articulation of three bones in the middle part of the ear assist in proper conduction of sound (Figure 1.2).

Morphometric investigation of these ossicles has been going since the mid-60s. Albeit the strategies are changing because of coming of more up to date advances and medicines. Jaw segments of vertebrates and columella auris of reptiles have delivered themselves to become Malleus, Incus and Stapes separately in developmental cycle. These 3 little bones are one of the substance of tympanic depression, which are sporadic, and laterally located in the center ear. They are an enunciated chain like structures interfacing horizontally with the tympanic layer and medially with average mass of the tympanic depression which leads the sound from tympanic film to cochlea through oval window (Kumar, Chaitanya & Singh, 2018). Knowing the morphometric life structures of ossicles has gotten vital in otologic medical procedures. In any ear pathology the sickness can dissolve the ossicles causing hearing misfortune (Dhingra & Dhingra, 2017).

Different abnormalities of these ossicles can prompt the hearing issues. Different intrinsic distortions of these ossicles have likewise been accounted for, to mess hearing up. To carry out the microsurgical moves and controls, in order to address these irregularities, specialists need to completely familiarize about the subtleties of these minuscule bones. Different abnormalities of these ossicles can prompt the hearing issues (Quesnel et al., 2015). Incredible level of ossicular disintegration is seen in cholesteatoma, bringing about helpless hearing result. (Gulati et al., 2019).

Different innate abnormalities of these ossicles have likewise been accounted for to mess hearing up. A few instances of intrinsic shortfall of a part of incus (long process) and capitulum of stapes have been accounted for. Nonappearance of this part of incus and positioning of stapes to malleus strangely can cause additional hearing misfortune. In some diseases, like Marfan's condition, microtia and Down’s syndrome different ontological distortions have been accounted for prompting combination of these ossicles and accordingly the consultation misfortune (Sodhi, Singh & Davessar, 2017).

The nearness of three interconnected sound-related ossicles in the ear is a characterizing normal for well evolved creatures, and parts of ossicle morphology are identified with hearing affectability. In any case, ossicles when it is investigated and examined, the difficulty level is a bit high due to their tiny size and intricate 3-D shapes (Stoessel, David & Gunz, 2016).

In intrauterine life, the ear ossicles begin to develop during the 4th week and it completely ossifies in the time period of 16 weeks and ultimately achieve their complete life-time size at birth (Saha et al., 2017). The varieties among people are fascinating organically, yet in addition it is significant as in respect of the methodology like ossiculoplasty and portable hearing assistant’s inserts after the birth (Purcell et al., 2016).

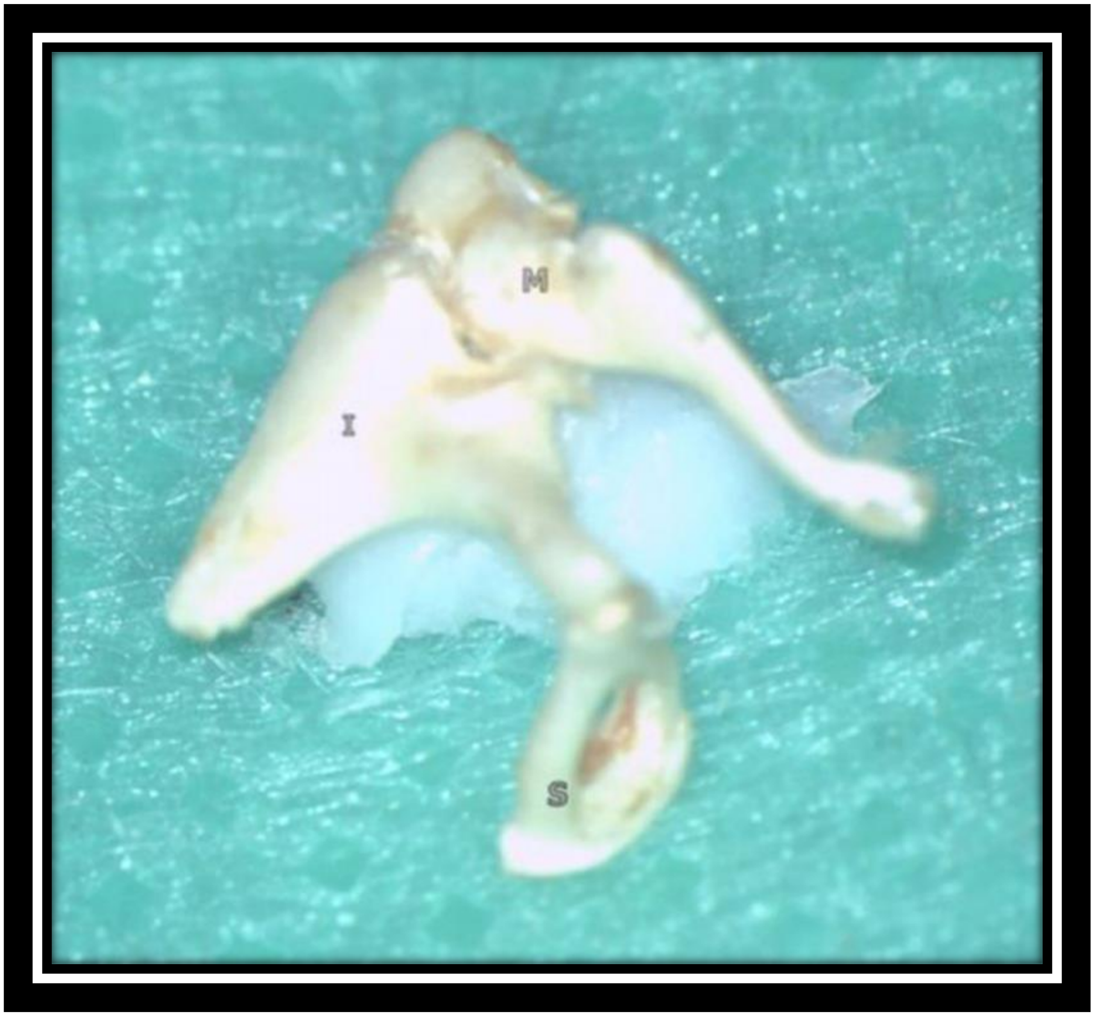


Figure 1.2 Middle ear ossicles. M-malleus, I- Incus, S- Stapes (Kumar et al., 2018)

1.1.2 PARTS OF EAR:

1.1.2.1 Outer part

Ear comprises of pinna and ear canal, the center part has tympanic film and ossicles and the inside sits on hard maze comprising crescent channels, utricles, saccule and cochlea. Hair and organs emit wax which are present in the outer part of the ear. This portion of ear offers safeguard and conduits sound. It is the visible part also known as auricle and pinna (Umar, Chaudhary & ur Rehman, 2017).

1.1.2.2 Center ear

It comprises of ossicles which is significant in light of the fact that it is occupied with various air spaces and this provides courses for disease to travel, Eustachian tube is likewise present here which adjusts the pneumatic stress among inward and external surfaces of ear drum (De Greef et al., 2015)

1.1.2.3 Internal ear

In order to maintain the body's equilibrium hearing organs are present which looks like a maze (Cochlea). It has an intricate shape which incorporates the hard and membranous maze (labyrinth). Snail-like cochlea is comprised of 3 liquid filled loads that twists around a hard center, which contains a focal chamber, an organ of corti which is a cochlear duct (Figure 1.3). The organ of corti has hair cells present in its inner surface which recognize sound and lead data through the cochlear nerve. External ear passes the waves to center part of the ear, and lastly arrive at the internal ear and its multifaceted organization of (nerves, channels, bones and cells) (Alberti, 2001).

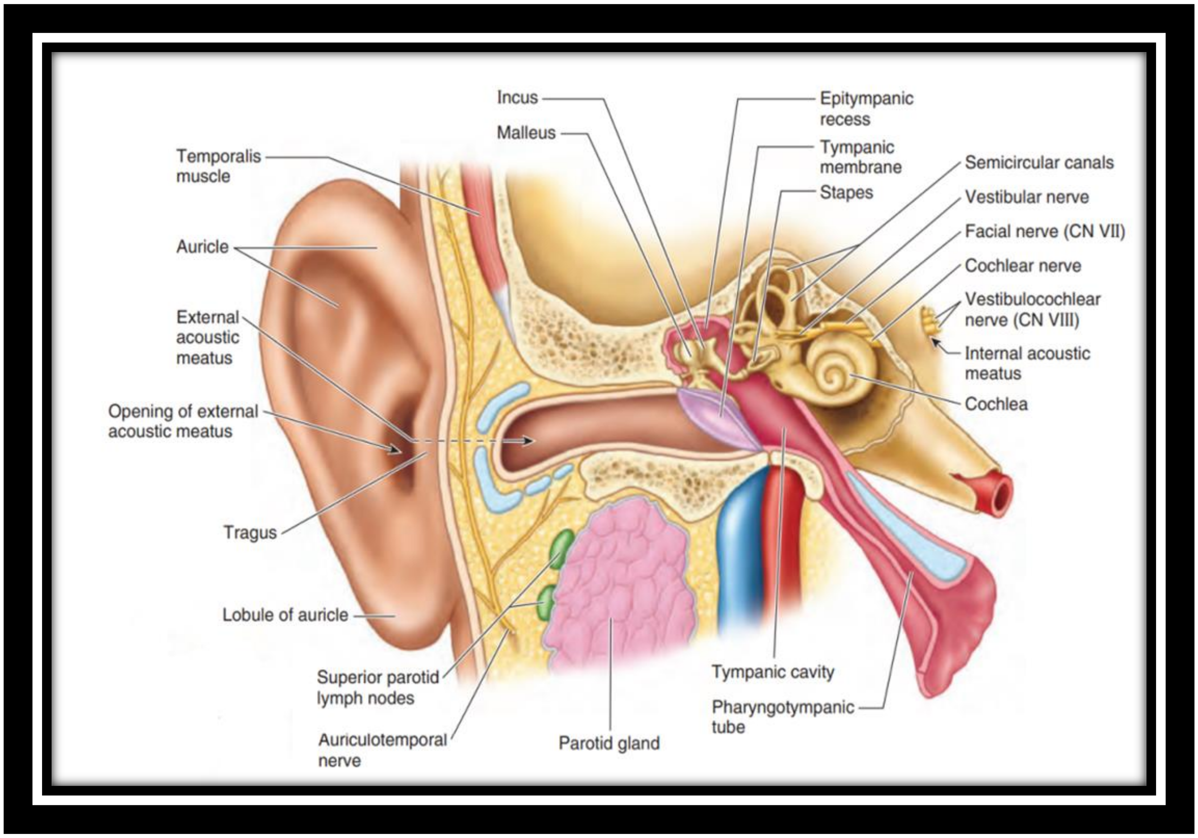


Figure: 1.3 Parts of the ear (External, middle and inner ear) (Moore & Dalley, 2018)

1.1.3 GROSS ANATOMY:

1.1.3.1 Outer Ear:

It has an auricle and an outer audible meatus. Auricle has a trademark shape and gathers air vibrations. It comprises of a flimsy plate of flexible cartilage covered by skin. It has both (extrinsic and intrinsic) characteristic muscles, which are provided by the facial nerve (Kappel, Makeig, & Kidmose, 2019). The outer audible meatus is a bended cylinder that leads from the auricle to the tympanic film. It conducts sound waves from the auricle to the tympanic layer (Niekrash, 2021).

The structure of the external third of the meatus is elastic ligament, and the inner 66% is bone, framed by the tympanic plate. The meatus is lined by skin, and its external third is furnished with hairs and glands (sebaceous and ceruminous). The last are adjusted perspiration organs that discharge a yellowish earthy colored wax. The hairs and the wax give a viscid wall that forestalls the passageway of foreign bodies (Lukolo, Kimera & Pilbee, 2021).

The tactile nerve supply of the covering skin is inferred from auriculotemporal and the auricular branch of the vagus nerve. The lymph seepage is due to the shallow parotid, mastoid, and cervical lymph hubs (Butt et al., 2020).

1.1.3.2 Center Ear:

The center ear is an air-containing hole in temporal bone (petrous part) and is fixed with mucous layer. It contains the ossicles, whose work is to send the vibrations of the tympanic membrane commonly known as (eardrum) to the perilymph of the inner ear. It is a thin, diagonal, slit-like pit whose long axis lies almost corresponding to the plane of the tympanic layer (Topsakal et al., 2021).

Center part of the ear has a rooftop, floor and four walls (anterior, posterior, lateral and medial). The rooftop is framed by a meager plate of bone, the tegmen tympani, which is a section of the petrous temporal bone. It isolates the tympanic hole from the temporal lobe and meninges of the brain. The floor is shaped by a slender plate of bone, which might be incompletely supplemented by a tissue of fibrous type. The superior bulb of the internal jugular vein is separated by this tympanic cavity through it (Atmaja, 2021).

Anterior wall of middle ear is framed underneath by a delicate plate of bone that isolates the cavity from the artery that is internal carotid supply. In the overhead compartment of this wall it open into 2 canals. The lower and bigger of these promote into the auditory cylinder, and the upper and shorter is the passage for a muscle (tensor tympani) into the canal. The delicate, hard septum, that isolates the canals, is drawn out on the medial wall in reverse, where it frames a shelf-like projection (Iskander, Naftalovich, & Pahlevan, 2020).

Tympanic membrane generally forms the lateral wall while the internal ear laterally shapes the medial wall. Mostly the wall shows a circular projection, known as promontory, which mainly is formed from the underlying 1st turn of the cochlea. Above and behind this projection lies the fenestra vestibuli, which is in oval shape and the base of stapes closes it. The window's medial side has the perilymph of the scala vestibuli of the interior ear. Underneath the back end of the projection lies the fenestra cochleae, which is round and shut by the secondary tympanic film. An adjusted edge runs on a level plane in reverse over the projection and the fenestra vestibuli and is known as the facial nerve channel prominence. On coming to the posterior wall, it bends and descends behind the pyramid (Bartling et al, 2021).

The tympanic layer is a fibrous, delicate layer that is magnificent dark. Membrane is diagonally positioned, facing lower, forward, and along the side. It is curved horizontally, and at the profundity of the concavity is a little despondency, the umbo is created by the tip of the malleus handle (Fig 1.5). At the point when the layer is enlightened through an otoscope, the concavity creates a "cone of light," which transmits inferiorly and anteriorly from the umbo (Snell, 2018).

Tympanic membrane is a delicate, thin, concave and semi-translucent wall present obliquely between the external and middle ear (Wahid & Nagra, 2018). Anatomically, this membrane is divided by malleolar folds (anterior and posterior) into two parts pars flaccida the upper small and pars tensa the lower larger part (Ediale, Adobamen & Ibekwe, 2018). It consists of three layers except in the upper part; cutaneous layer (outer) which is in continuity of the skin, fibrous layer (middle) has both circular and radial fibers while a layer of mucous membrane (inner) in its innermost surface (Adegbiji et al., 2018).

The tympanic membrane surface area is 64.3mm^2 which amplify the sound by 18.3 dB (Gaur et al., 2017). Its diameter is approximately 10mm vertically and 5mm horizontally (Wahid et al., 2018). Today, in a human being, a value of thickness uniformly across the whole membrane ranges from (30–150 μm), that is mostly considered by many authors (Aernouts, Aerts & Dirckx, 2012). While the thickness of membrane varies in different parts of membrane that is pars tensa, central region of the membrane and towards manubrium (Buytaert et al., 2013)

The middle ear includes three small bones that are malleus, incus and stapes. In the tympanic cavity it forms a link between the oval window medially and tympanic membrane laterally (Quam, Coleman & Martinez, 2014).

The most laterally in the cavity is present a hammer like bone called malleus. It has a head, neck, anterior process, lateral process and manubrium also known as handle. Incus appears as anvil and has a short process, a body and a long process. In incus at the long process tip, a small mass is present known as lenticular process. This process articulates with the stapes head which is the smallest of all bones in the body that appears like a little stirrup and consists of a small head on top, two crura, a base also known as footplate and a neck. Malleus articulating surface is in contact with the body of the incus by a synovial joint (saddle type). Stapes head articulates with the lenticular process and forms an ‘incudostapedial’ joint (Saha et al., 2017).

Clinically two important muscles are present in the center part of the ear (stapedius and the tensor tympani). In humans the tiniest muscle in the body is the stapedius, which attaches to the stapes bone and it is innervated by the seventh cranial nerve (facial). Malleus provides attachment to the second muscle that is tensor tympani which is supplied by a mandibular division of fifth cranial nerve (Figure 1.4). Acoustic reflex is mainly generated by these muscles, which helps to soften the sound that is being transferred to organ of corti, a structure present in the inner part of the ear and reduces its damage. It is of great importance that during the surgical measures, the surgeon needs to maintain the integrity of facial nerve which has numerous branches in the vicinity of central ear as it could lead to the paralysis of one side of the face (Green, Shelton & Brackmann 1994).

There are two muscles that are present in the center part of the ear, the tensor tympani that originates from the wall of auditory tube and from its own canal inserts at malleus handle supplied by the Mandibular division of cranial nerve V (trigeminal nerve) that dampens down vibrations of tympanic membrane while the 2nd muscle is stapedius that originates from the pyramid (bony projection on posterior wall of middle ear) and inserts at the stapes neck supplied by the cranial nerve VII (Facial nerve), it dampens down the vibrations of stapes (Pagano et al., The FASEB Journal, 35).

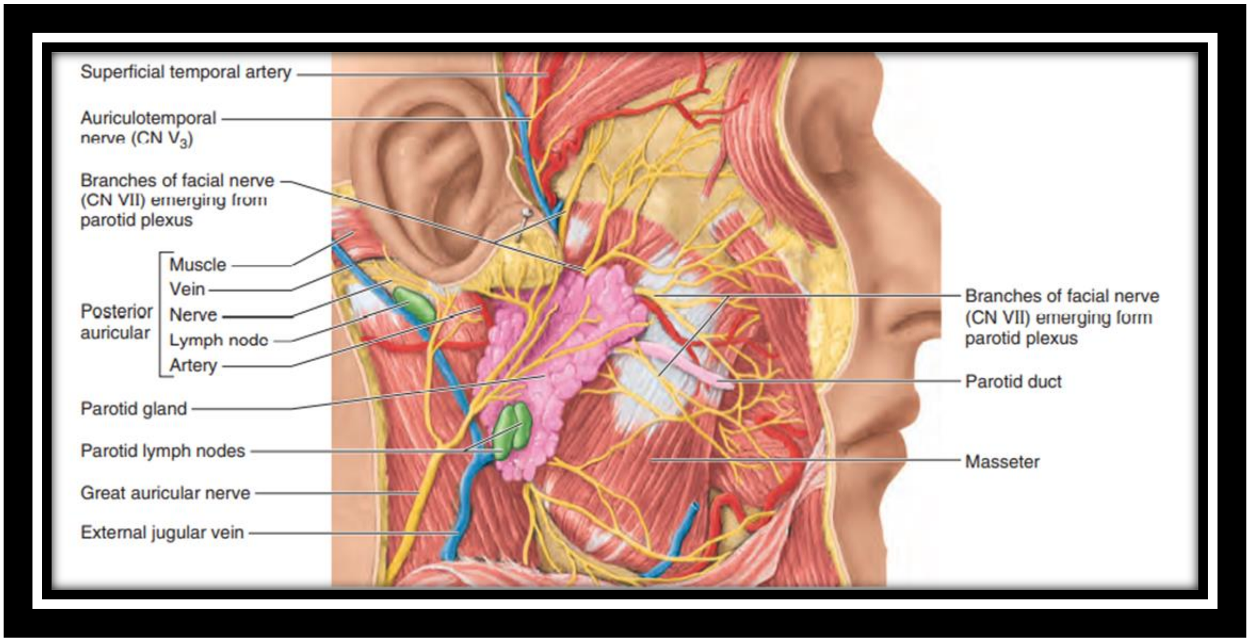


Figure: 1.4 Neurovascular supply of the ear (Moore & Dalley, 2018)

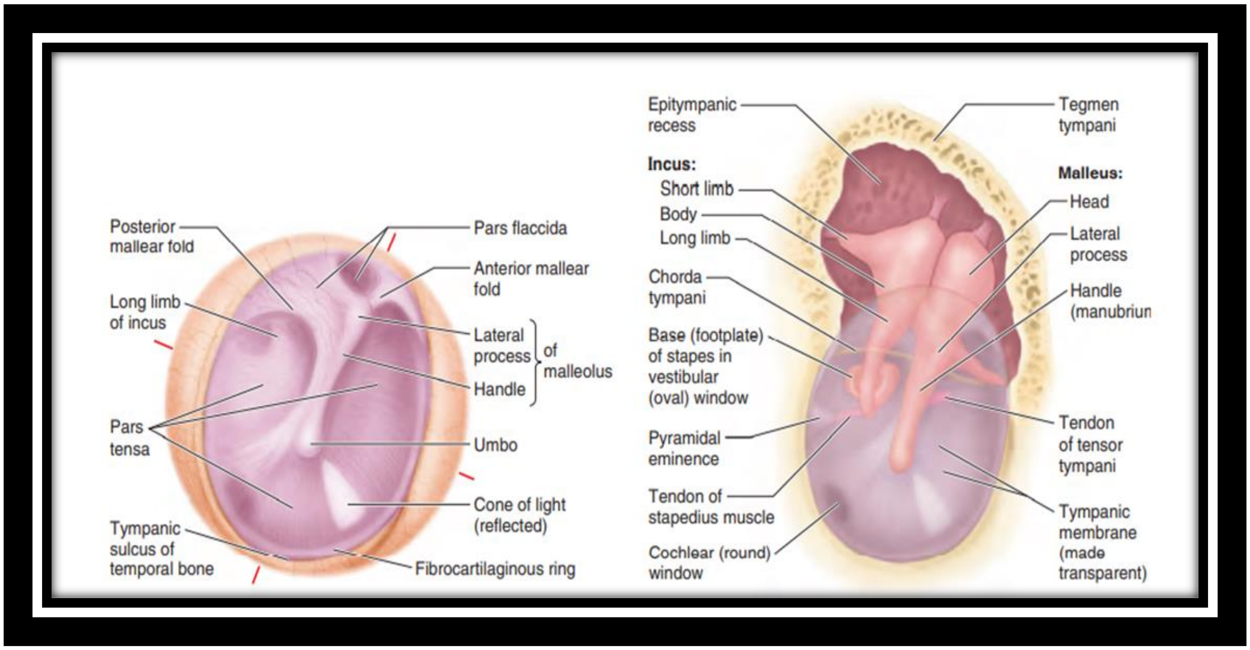


Figure: 1.5 Tympanic membrane in contact with ear ossicles (Moore & Dalley, 2018)

1.1.3.3 Inner ear:

It comprises of two labyrinth (bony and membranous). The bony one comprises of three sections: the vestibule, the crescent trenches (semicircular canal), and the cochlea. These are depressions arranged in the substance of thick bone. They are surrounded by endosteum and enclose a clear liquid, the perilymph, wherein is suspended the membranous one. The central part of this labyrinth is the vestibule, which is present behind the cochlea and in front of the semicircular canals. The fenestra vestibuli is present in the lateral wall and the stapes base closes it. The secondary tympanic membrane closes the fenestra cochlea and the annular ligament. The utricle and saccule of the membranous labyrinth are located within the vestibule (Lim & Brichta, 2016).

The superior, lateral and posterior canals that are semi-circular in shape open into vestibule's posterior part. Ampulla is a swelling present at the ends of each canal. Semicircular ducts are present in each canal (Figure 1.6). A snail resembling structure, cochlea is present in vestibule's anterior part. The membranous one is located within the bony labyrinth. Endolymph fills this labyrinth and perilymph surrounds it. Bony vestibule has a saccule and utricle (Iversen & Rabbitt, 2017).

The rotation of malleus and incus bone is on the anteroposterior axis that goes through the ligament in communication with the malleus (anterior process) to the tympanic cavity's anterior wall, the malleus (anterior process) and the incus (short process) and connection of the incus process with the tympanic cavity's posterior wall is provided by the ligament. Medial movement of the tympanic membrane also causes the malleus handle to move medially and malleus head and incus body laterally. Medial movement of incus long process with stapes. In the fenestra vestibuli, stapes base is pushed medially and the motion is connected to the scala vestibuli perilymph. Due to the characteristic of being incompressible the liquid (perilymph) causes secondary tympanic membrane to bulge out in outward manner in cochlea (fenestra) at scala tympani inferior end (Snell, 2018).

The nerve of tympanic part of the ear emerges from the glossopharyngeal nerve, beneath the foramen called as jugular. It goes over the base of the center ear and onto the projection. Here it divides into subdivisions, which structure the (tympanic plexus). This plexus supplies the coating of the center ear and radiates the lesser petrosal nerve, which sends secretomotor filaments to the parotid organ by means of the otic ganglion (Figure 1.7). Then it joins the ganglion as it leaves the skull through ovale foramen (Vayisoğlu et al., 2021).

In internal acoustic meatus when it reaches to its lower part, it isolates into vestibular and cochlear parts. The nerve known as vestibulocochlear is extended to shape the vestibular ganglion. The parts of the nerve at that point penetrate the lateral end of this meatus and get access to the membranous maze, where they supply (the utricle, saccule, and semi-circular ducts ampullae) (Walijee et al., 2021).

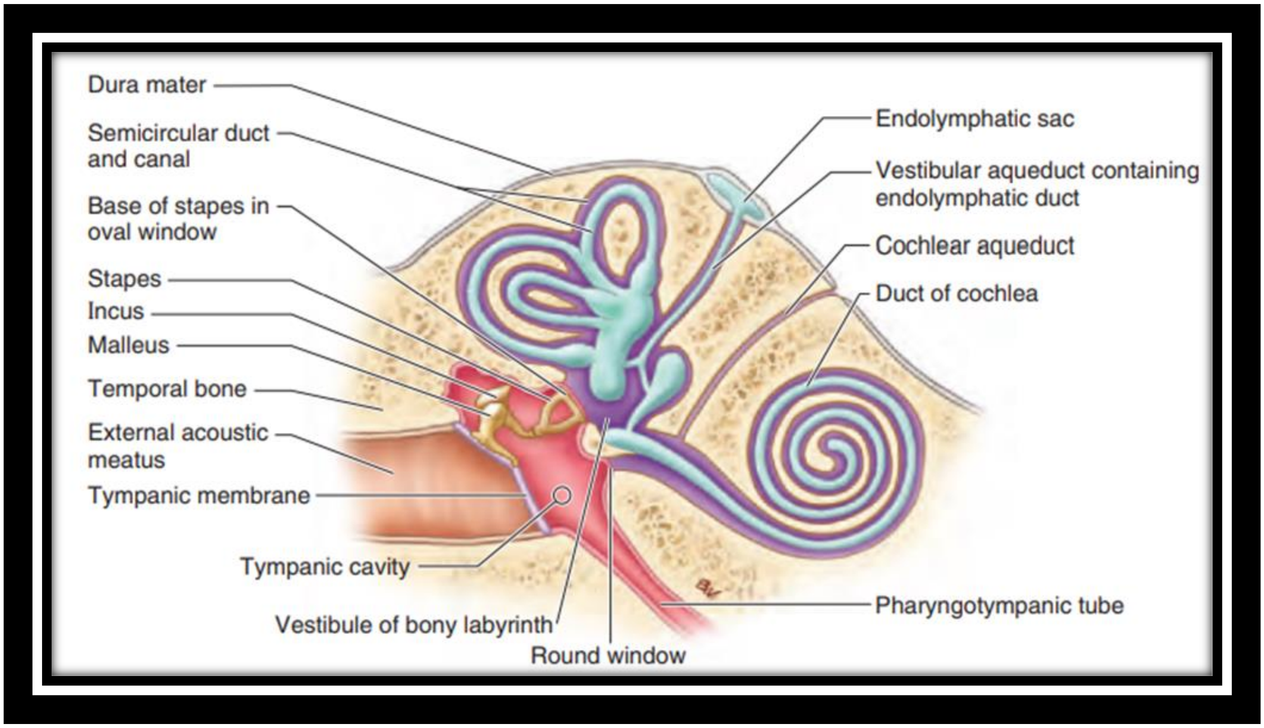


Figure 1.6 Organ of hearing Cochlea (Round and oval window) (Moore & Dalley, 2018)

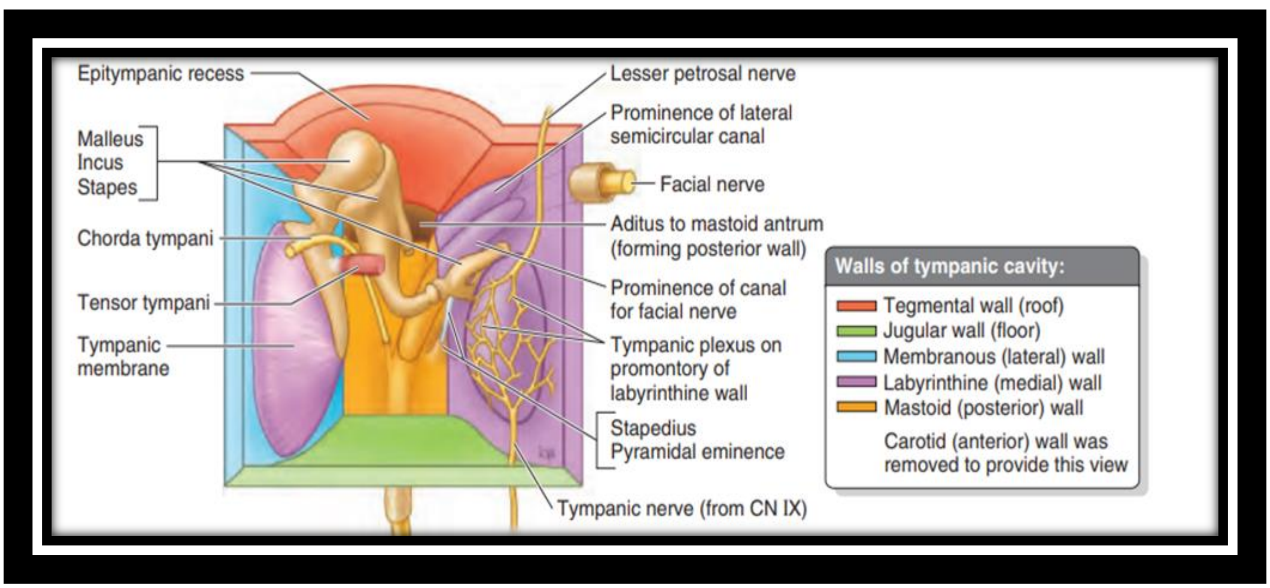


Figure 1.7 Walls of tympanic cavity (Moore & Dalley 2018)

1.1.4 DEVELOPMENT OF EAR:

The ear develops from first pharyngeal (branchial) pocket and six minuscule swellings that twist in the underlying undeveloped organism known as otic placodes (from ectoderm). It is made from three anatomic portions: outer ear, comprising of the pinnae (auricle), auditory meatus and eardrum. Center part of the ear comprises of three little hear-able ear bones named as ossicles, and the tympanic layers present inside, that are associated with the oval windows. Internal part of the ear comprises of the organ (Vestibulocochlear), which is apprehensive about audition and equilibrium. Parts of the ear that transfer the auditory signals from the outside to the inner ear transform these signals to nerve stimulating forces and detect variations in harmony which are driven by outer and middle part (Sundar, Chowdhury & Kamarthi, 2021).

1.1.4.1 Inner ear:

This is the first part that develops in the ear. As the 4^{th} week starts, an ectoderm is thickened (surface), forming a placode (otic) and appears up on both sides of the caudal part of the hindbrain, the myelencephalon (Figure 1.8). Placodes develop from the surface ectoderm which receives stimulatory signals from the notochord and paraxial mesoderm. Soon invagination of each otic placode sinks profound to the surface ectoderm into the fundamental mesenchyme. In this manner, otic pit develops. After joining of pit edges, it shapes into an otic vesicle (membranous labyrinth's primordium). The otic vesicle before long loses its association with the surface ectoderm, and a diverticulum develops from the vesicle and prolongs to shape the endolymphatic pipe and sac. (Moore, Persaud & Torchia, 2018).

Otic vesicles from which the utricle, little channels of endolymph, and crescent conduits emerge. Anterior parts of saccule, which offer ascent to the conduits of saccules and cochlear. Three in number and shaped like circles, these diverticula develop from the parts of utricle of the membranous mazes (labyrinth) at their early stages (Figure 1.9). Before long the focal pieces of these diverticula combine and vanish. The unfused fringe portions of the diverticula become the half circle conduits, which are connected to the utricle and are subsequently encased in the crescent channels of the hard maze. Confined dilatations, the ampullae, create toward one side of each crescent pipe. Specific receptor zones present in the ampullae called the ampullares of cristae differentiate into vestibular system (Mackowetzky et al., 2021)

Cochlear pipe develops anteriorly from the saccular region (round diverticulum) of the otic vesicle and curls to frame the membranous cochlea. Formation of the ductus reuniens soon occurs which is an association of saccule with the cochlea (Figure 1.9). The pipe of cochlea and its cells are separated from the twisted organ (of Corti). Ganglionic cells of the CN VIII nerve traverse along the curls of the membranous portion of cochlea and make the cochlea's spiral shaped ganglion. Processes of nerve stretch out to the spiral organ from the cochlear ganglion, end on their hair cells. Cells of the spiral ganglion hold their undeveloped bipolar state. Preparatory signals from the otic vesicle trigger the mesenchyme around it to gather and distinguish into an otic capsule made up of cartilage (Mammano, 2018).

The perilymph contains the membranous maze. Scala tympani and scala vestibuli are two divisions that branch out from the perilymphatic cavity (Figure 1.9). The internal ear's bony labyrinth is formed by the ossification of the otic capsule's cartilage. By the mid-fetal period (20-22 weeks) the internal ear attains its full shape and size (Costeur et al., 2017).

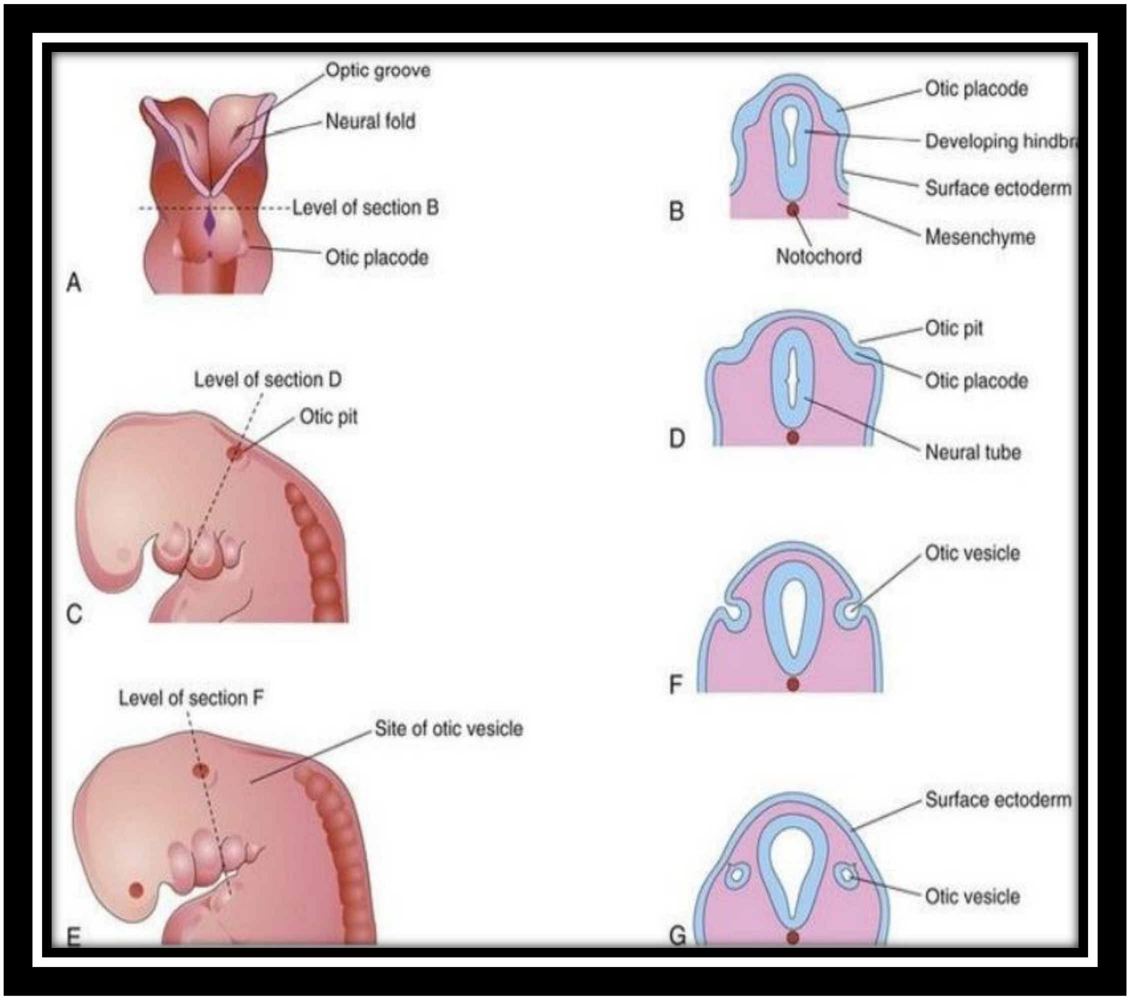


Figure 1.8 Drawings above illustrate the early development of the internal ear. **A**, Dorsal view of an embryo at approximately 22 days, showing the otic placodes. **B, D, F,** and **G**, are the schematic coronal sections illustrating successive stages in the development of otic vesicles. **C** and **E**, Lateral views of the cranial region of embryos, at approximately 24 and 28 days, respectively. (Moore, Persaud & Torchia, 2018).

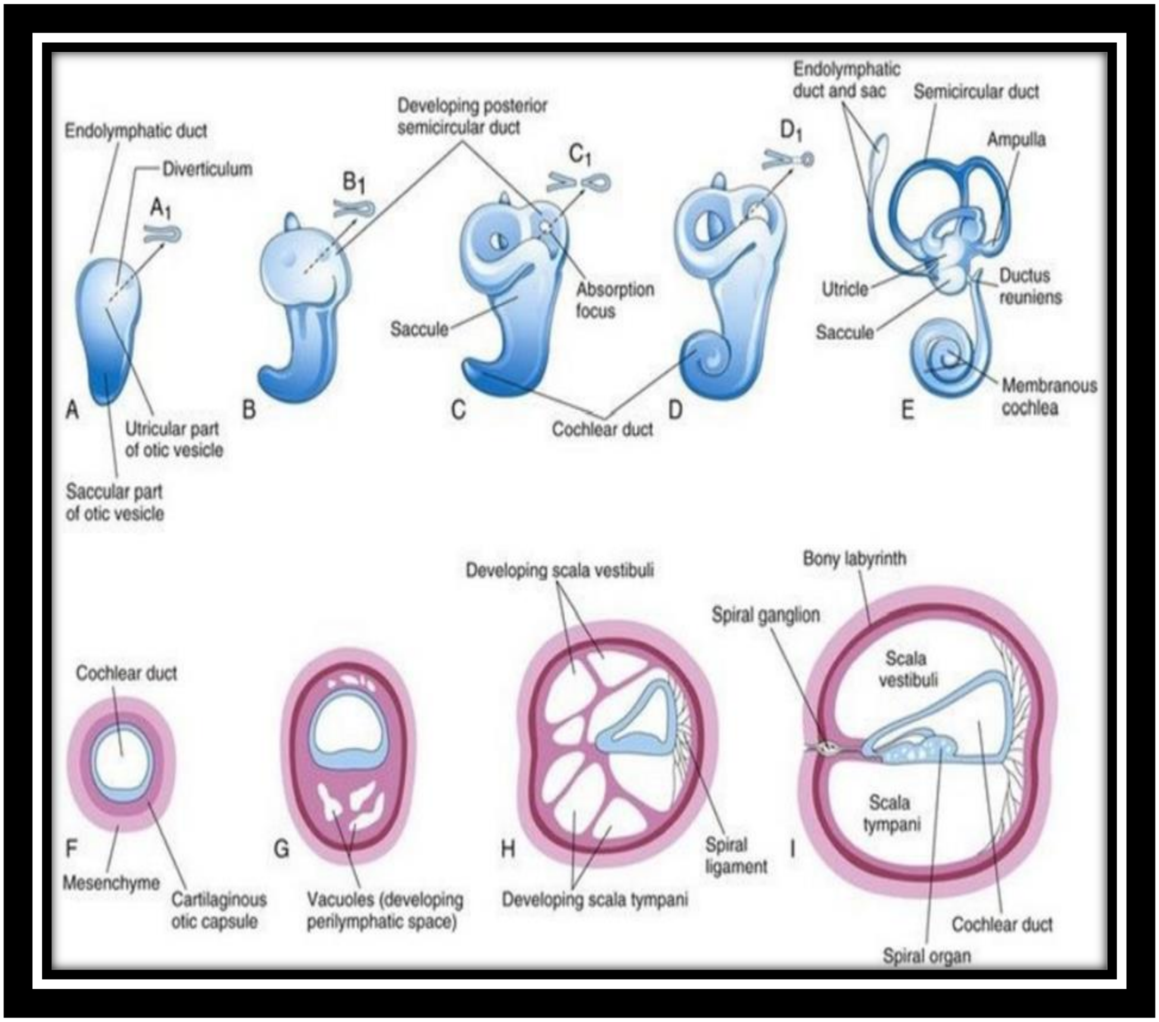


Figure 1.9 Drawings of the otic vesicles showing the development of the membranous and bony labyrinths of the internal ear. **A** to **E**, Lateral views showing successive stages in the development of the otic vesicle into the membranous labyrinth from the fifth to eighth weeks. **A** to **D,** Diagrammatic sketches illustrating the development of a semicircular duct. **F** to **I**, Sections through the cochlear duct showing successive stages in the development of the spiral organ and the perilymphatic space from the 8th to the 20th weeks. (Moore, Persaud & Torchia, 2018).

1.1.4.2 External ear

The first pharyngeal groove gives rise to the external auditory meatus from its dorsal part, which is a passageway to the tympanic membrane. Present at the end of this funnel-shaped conduit are the cells of the ectoderm which multiply and form the meatal plug, a solid plate of epithelium. Towards the late fetal period, disintegration of the plug's central cells produces a cavity that forms the inner part of the external auditory meatus (Figure 1.10). The meatus is comparatively short at birth but by the ninth year it achieves its adult length (Anthwal & Thompson, 2016).

Encircling the first pharyngeal (branchial) groove are the auricular hillocks. The latter is a mesenchymal propagation in the first and second branchial arches which forms the pinna (auricle), lateral projections from the sides of the head. The part of the pinna which develops in the last, is the lobule (earlobe). Development of the auricles is at the base of the neck and their normal positioning depends on the development of the mandible (Moore, Persaud & Torchia, 2018).

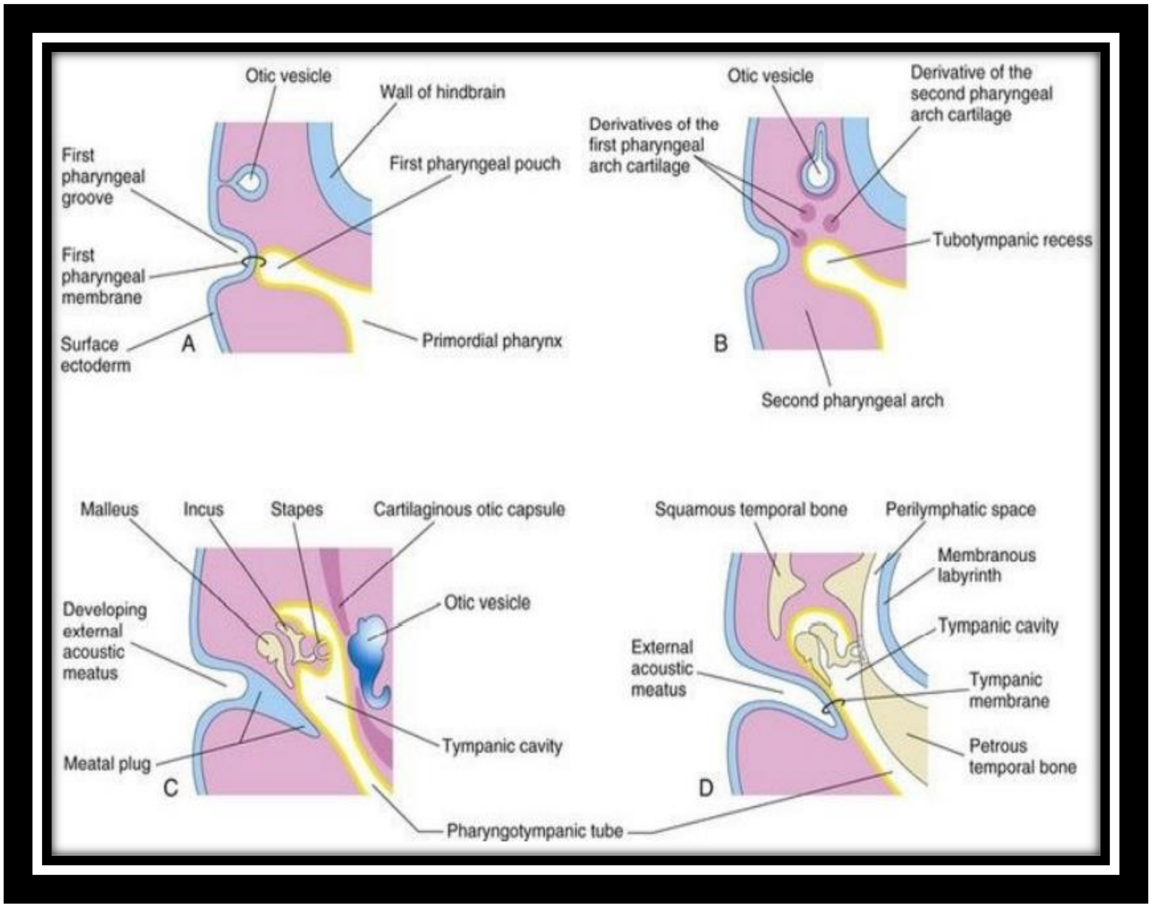


Figure 1.10 Schematic drawings illustrating development of the external and middle parts of the ear. **A**, At 4 weeks, illustrating the relation of the otic vesicle to the pharyngeal apparatus. **B**, At 5 weeks, showing the tubotympanic recess and pharyngeal arch cartilages. **C**, Later stage, showing the tubotympanic recess (future tympanic cavity and mastoid antrum) beginning to envelop the ossicles. **D**, Final stage of ear development showing the relationship of the middle ear to the perilymphatic space and the external acoustic meatus. (Moore, Persaud & Torchia, 2018).

1.1.4.3 Middle ear

The outer surface of tympanic membrane is formed by the first pharyngeal membrane, the former's primordium. In the embryo, the separating medium between the first pharyngeal groove and the first pharyngeal pouch is the tympanic membrane. As development progresses, the two parts of tympanic membrane are infiltrated by the mesenchyme which differentiates into the collagen fibers of the tympanic membrane. The tympanic membrane develops from mesenchyme of the 1^{st} and 2^{nd} branchial arches, ectoderm of the 1^{st} branchial groove and endoderm of the tubotympanic recess (Figure 1.10).

Growth of ear ossicles starts dorsally from principal pharyngeal curve (Meckel cartilage) is firmly identified with the developing ear. Initially during development, little knobs split away from the proximal piece of this cartilage and structures two of the center ear bones, the malleus and incus. The center part of the cartilage relapses, yet its perichondrium structures the front tendon of malleus and the sphenomandibular tendon (Moore, Persaud & Torchia, 2018).

1.1.5 HISTOLOGY OF EAR:

Cartilage basically plays a vital role in the formation of many parts of the ear which is a type of a connective tissue, an intense, strong type of supporting tissue, described by an extracellular network (ECM) with increased quantity of GAGs and proteoglycans, collaborating with collagen and flexible strands. Primary highlights of its lattice make cartilage ideal for an assortment of mechanical and defensive parts inside the grown-up skeleton and somewhere else. Elastic cartilage is more flexible than hyaline cartilage and it is found in ear auricle, in outer audible meatus, in auditory (Eustachian) tubes and outer canals (Figure 1.11). ECM has main structures that contains collagen (type II), elastic fibers (dark) and aggregan. Mostly it consists of chondroblasts, chondrocytes and perichondrium (Mescher, 2013).

Stratified squamous (keratinized) type of epithelium lines the outer ear that includes auricle and canal (Hentzer, 1970).

The sound energy is concentrated and vibrates in ear canal to membrane through peripheral ear. The peripheral ear canal divides into a lateral and medial part which consist of elastic cartilage (laterally) and bone. Glands (apocrine) that produce ear wax inside the canal which is essential for the prevention of any infection. Conversely, excessive amount of wax can block the canal and aggravate loss of hearing leading to conductive type of loss. The sensory supply of the outer ear is from numerous nerves, comprising of the greater auricular nerve, lesser occipital nerve, auriculotemporal nerve a branch of the mandibular nerve, and branches of the facial and vagus nerve. Induction of a nerve can cause a clinical representation of a cough reflex that happens due to (auricular branch of the vagus nerve) (Tekdemir, Aslan & Elhan, 1998).

The outer ear is separated by tympanic cavity by a structure known as tympanic membrane. It is connected with skin externally lined by stratified squamous keratinized epithelium and has internally a mucous membrane basically lined by a stratified squamous epithelium (non-keratinized). Laterally this membrane is innervated by trigeminal and vagus while medially by glossopharyngeal nerves (Uddman et al., 1988).

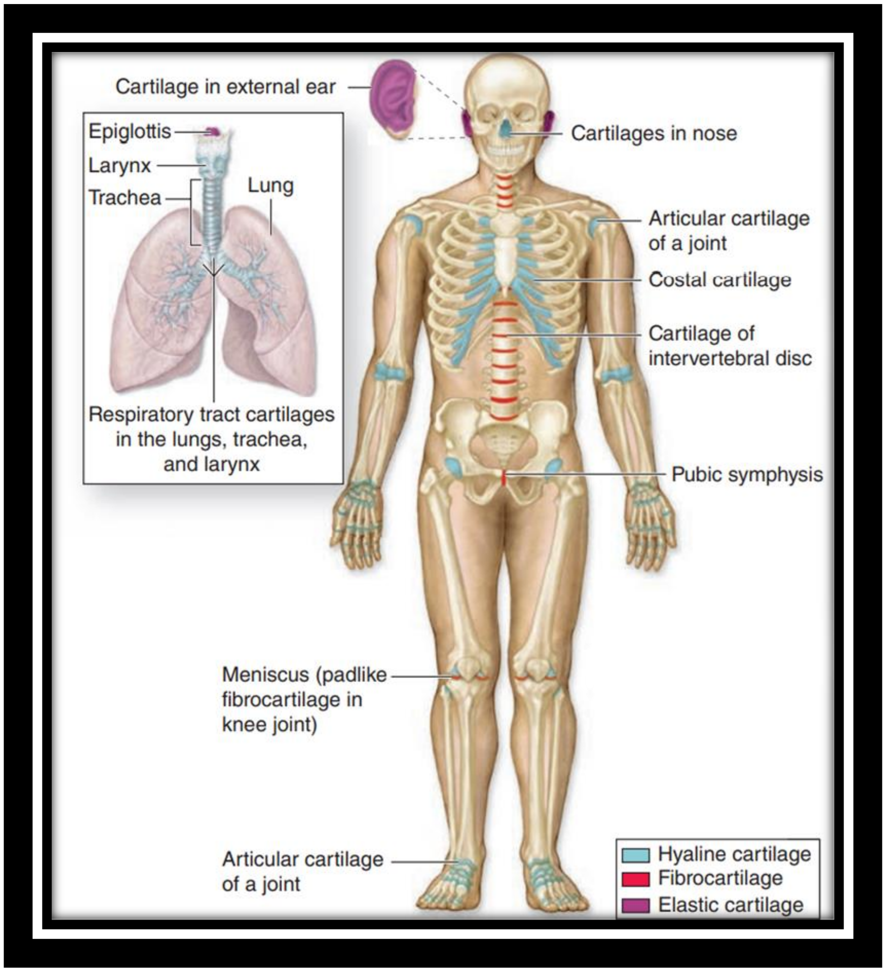
Starting from the membrane the center part of the ear extends to the window that is oval in shape present in the inside part of the ear, which is covered by stratified squamous epithelium non- keratinized (Tekdemir et al, 1998).

1.1.5.1 Microscopy Light:

Visualizing an ear specimen via light microscope, numerous elements are detectable. A cross-sectional representation of outer ear will display a stratified squamous keratinized epithelium beneath that is a cartilaginous structure and wax-manufacturing glands, which may be noticeable. Stratified squamous epithelium of a non-keratinized type is present in the center part of the ear. Under the microscopic representation internal part of the ear has been found to be having the most intricate structure. Cross-sectional view of cochlea shows a spiral ganglion, bone, organ of corti and two fluid-filled channels. Inner part of the ear consists of extremely particular type of cells that comprise of pillar, hair, Claudius, Boettcher, Deiters cells and spiral ganglion neurons (Engström, Ades & Hawkins, 1964).

Outer audible meatus, pinna, ear wax and cavity of tympani are parts of outer ear. In which pinna and outer ear canal has a skin lined with keratinized stratified squamous epithelium (Christov et al., 2020).

Eustachian tube that is present in the middle part of the ear is lined with ciliated simple columnar epithelium and changes to pseudostratified ciliated columnar and also consists of goblet cells near to the pharynx. Flat, thin, singular layer of cuboidal epithelium lines the tympanic membrane. The internal part of the ear has a columnar epithelium covered by vascular stroma in the labyrinth (vestibular). (Ansen et al., 2020)



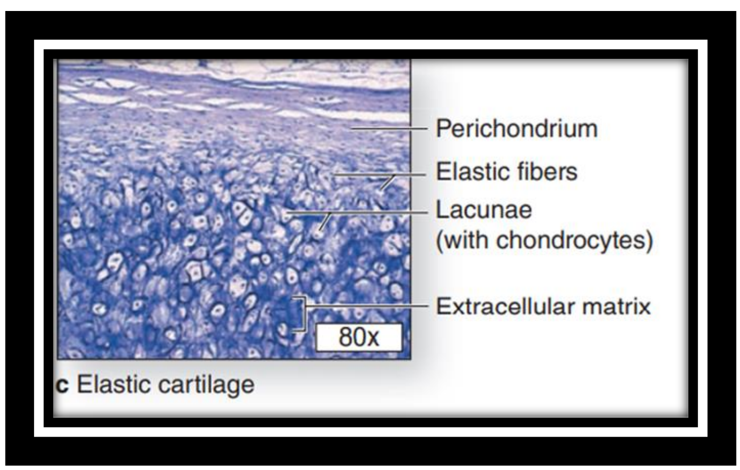


Figure 1.11 Elastic cartilage present in the ear (Mescher, 2013)

1.2 Hypothesis

A) Null hypothesis:

There are no notable variations in the morphology of ear ossicles in every individual and it has no correlation with age and hearing thresholds.

B) Alternate hypothesis:

There are notable variations in the morphology of ear ossicles in every individual and it has a correlation with age and hearing thresholds.

1.3 Objective(s) of study

A) To determine the morphological variation in ear ossicles

B) To determine the correlation of these variations with age, gender, ethnicity and hearing thresholds.

C) To investigate the variations of ear ossicles on CT- scan.

1.4 Problem statement

Morphological variation in ear ossicles is still a matter of discussion and there are different views and arguments whether these bones remain static and reaches to its definite size and form before birth or shows variation in morphology in adult age. In human beings, referring to the auditory ossicles, some scientists concluded that it reaches to an absolute dimension and form in fetal periods and postnatal alterations are negligible, whereas there are certain studies which oppose this claim. These claims provoked us to make an attempt to discover the developmental pattern of the ossicles and linking the facts with adults. Similar findings were reported that auditory ossicles reach their adult size in fetus by 28 weeks of intrauterine life (IUL) (Nadeem, 2012). A study reported that the development of auditory ossicles in humans is not completed during fetal life. The different parameters in the fetal periods increased adequately in the post-natal life (Rolvien et al., 2018).

The importance of this examination is to give new anatomic record in regard to this structure in addition to with the assistance of the boundaries taken in this investigation, we will portray a potential clarification for the most part for great hearing outcomes, we will show constraints for the sign of explicit prosthesis as a result of an incredible variety of distances across, not referenced in writing before with assistance of a CT check. Any variations undetected may cause the surgeon to misinterpret the actual form and size of these bones leading to improper prosthesis and mishap in surgeries related to this structure. Due to lack of knowledge regarding its morphology it is difficult to conclude its consistency in form, throughout an individual’s life. So for its better understanding it needs to be evaluated thoroughly.

Keeping in mind this issue, with the help of HRCT of petrous temporal bone surgeons can identify anatomical variations and avoid any irregularity in surgeries and provide better quality of hearing.

1.5 Significance of study

First time in Pakistan in this study, humans CT scans will be used to enlighten not only the new dimension of information regarding the anatomical variations in ear ossicles but also will increase the horizon to be able to measure these changes on CT scans rather than going into the cadaveric study which consumes time for obtaining such precious information.

Occupancy of such variations is considered to straightforwardly affect the pre-surgical assessments, those while in transit to surgery. It is normal that this work may likewise rouse numerous others to proceed with CT (petrous temporal bone) so as to assemble more data in regards to ossicles which may be valuable according to convention later on systems of homografts in ossiculoplasty. The information on morphology is basic for any prosthesis system so as to forestall putrefaction of the concerned structure because of pressure of taking care of veins.

CT scanning is a painless, special x-ray equipment that is used to evaluate these variations. CT scan must be performed after complete ear examination. This prospective study will help to determine the incidence of morphological variants in ossicles and assess its association with age, gender, ethnicity and hearing thresholds.

1.6 Operational definitions

1.6.1 Ear ossicles (EO)

They are the tiniest bones in the human body. Inside the tympanic pit, the three center ear ossicles (malleus, incus and stapes) structure a hard connection between the tympanic film along the side and the oval window medially (Quam et al., 2014).

1.6.2 Computed tomography (CT) Scan

It is a type of structural imaging technique in which x-ray are used to create tomography that is cross sectional images. The procedure of this technique involves patient lying onto the scanner table and then pass through the CT gantry, in which x-ray tube is present and oppositely located array of x-ray detectors are present which rotate about the patient. Large number of x-ray projections are attained from multiple angles at each slice position in the patient, each of which contains data regarding the differential attenuation of x-rays by different tissue type in the patient. These projections are then used by the computer to reconstruct CT images (Torigian & Ramchandani, 2018).

1.6.3 Pure tone audiometry (PTA)

It can decide the sort and amount of hearing misfortune by deciding pure tone thresholds both for the air and bone conduction (Anwar, Khan & Khan, 2019).

1.6.4 Otoscope

It is used for ear examination (Wahid & Nagra, 2018).

1.6.5 Tympanometry

It is utilized to test normal working of middle ear before continuing to have audiometry (Ediale, Adobamen & Ibekwe, 2018).

1.6.6 Audiogram

It gives data as to hearing affectability across a chosen range of frequency (Musiek et al., 2017).

1.6.7 Decibel (dB)

It is a value that is utilized to demonstrate how people hear a given sound (Giuliano & Pozzar, 2021).

CHAPTER 2

LITERATURE REVIEW

Ear ossicles are minute three paired bones named as Malleus, Incus and stapes, present in the middle part of the ear. It has an essential function in means of hearing. Ever since mid-60s, morphometric analysis has been carried out on the ossicles of the ear. Due to their miniature size, the information on it is scarce and difficult to record despite the evolution of techniques, advancements, and medicines. Measurements of these bones when taken into account, there were different methods used in various studies depending on the inclusion of the subjects as it could be the cadavers or a living subject.

Diverse techniques were used for the morphometric analysis of the ear ossicles that has been recorded in the literature starting with the readings taken in this study that included 60 arrangements of ossicles from transient bones. The ossicles were acquired by 'canal wall down' mastoidectomy procedure. After acquiring and cleaning the ossicles, the gathered ossicles were cleaned, and picture photographed, under 6.4 x amplifications utilizing a microscope (LEICA) 320, with a goal of 2048 × 1098 pixels (magnification). A software names as (Fiji) was used to estimate each photograph by setting the scale to millimeter (mm). Morphometric information of ossicles helped in analyzing all the data. Distorted ossicles were prohibited from study. The parameters taken into consideration mentioned in (Figure 2.1, 2.2 and 2.3) (Kumar et al., 2018).

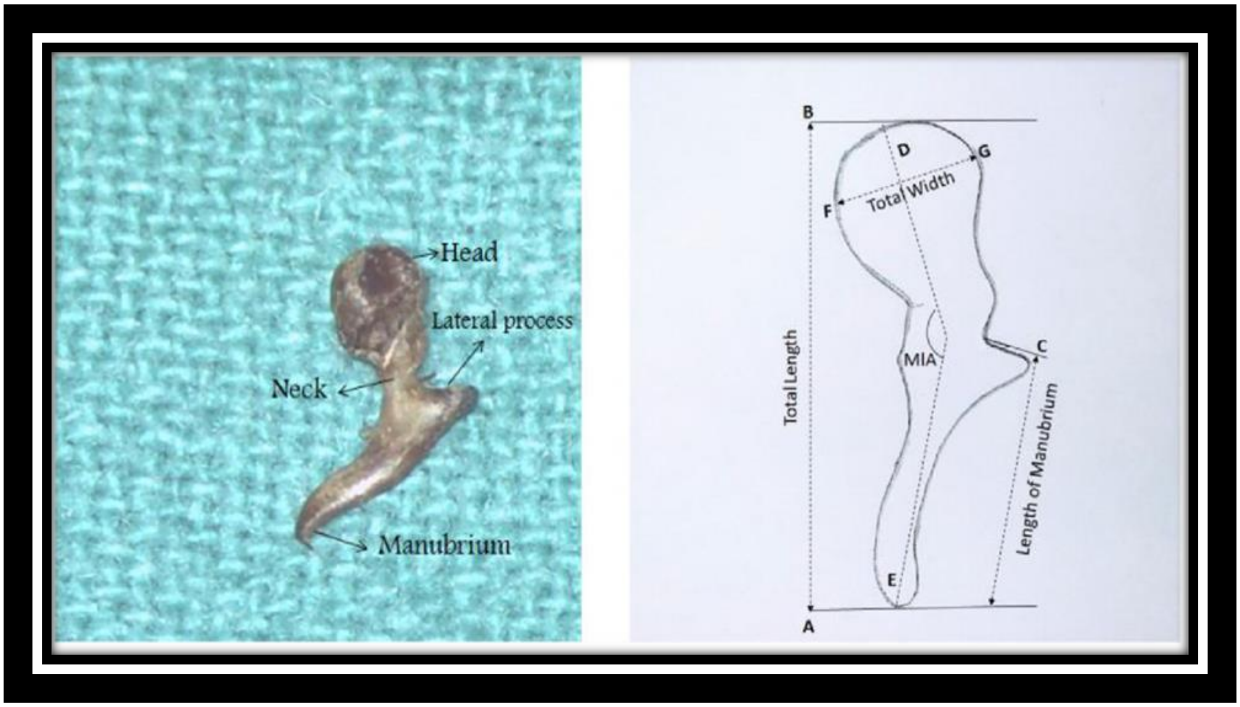


Figure 2.1 Landmarks of malleus [Malleus A-B (Complete length): Largest distance between the highest point of the head and manubrium distal part, C-A (Manubrium length): Largest distance from the upper edge of the lateral process than the distal finish of the manubrium, D-E (Angle MlA): Measured between the long pivot of the neck and manubrium, F-G (Complete Width of head): Greatest width of the head] (Kumar et al., 2018).

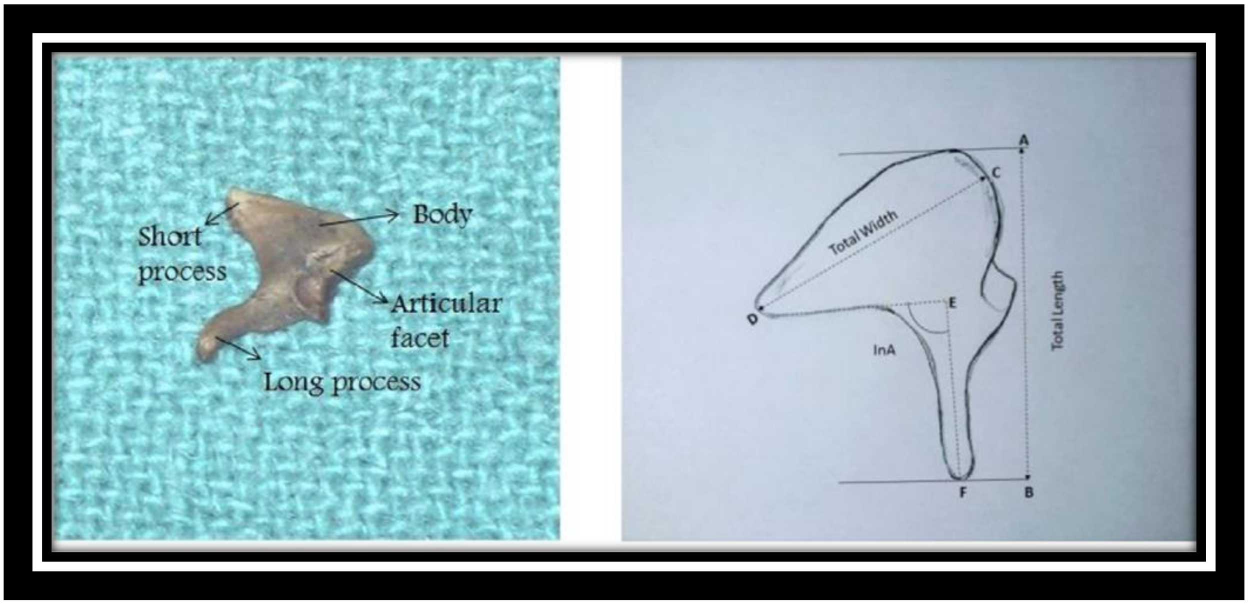


Figure 2.2 Landmarks of incus [Incus A-B (Complete length): Largest distance between the prevalent edge of the body and the long process (distal end), C-D (Complete width): Largest distance between the short process tips to the most projecting piece of the (articular facet), E-F (Long process complete length): Largest distance between the upper edge and the long process (distal end), InA (Angle): Measurement taken between the lower edge of the short process and dorsal edge of the long process] (Kumar et al., 2018).

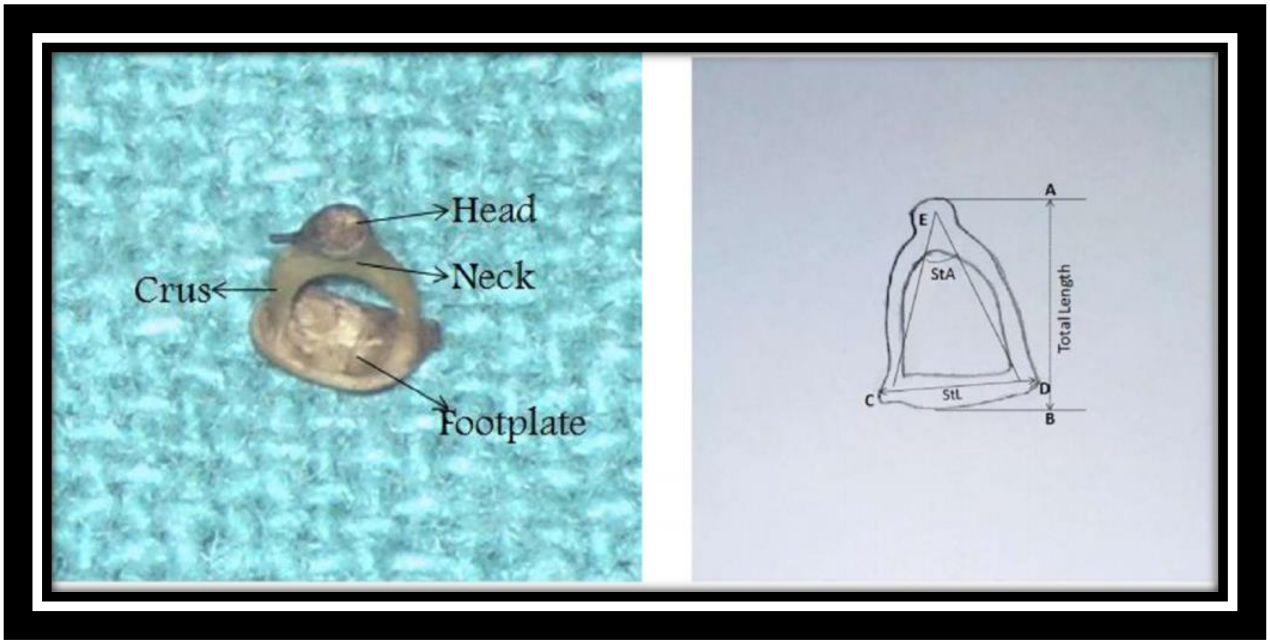


Figure 2.3 Landmarks of stapes [Stapes Total height A–B (Total Height): maximum distance between the top of the head to the footplate, C-D (Total length of the footplate StL): maximum distance of the long axis of the footplate, Width of the footplate: maximum distance of the inner aspect of footplate in short axis, E (Angle StA): measured between the two crura of the stapes (i.e., Line drawn by connecting points at the junction of crura i.e., neck of stapes (E) and (C-D) footplate on either side] (Kumar et al., 2018).

In another study a simple microscope was used to analyze the morphological changes in the ossicles. 100 arrangements of center ear ossicles with 50 left and 50 right side of the ear, each set comprising of (malleus, incus and stapes) were gathered from 100 corpses accessible among the different clinical universities of North India. The bones were meticulously analyzed under the basic microscope and results mentioned that stapes was discovered to be most variable ossicle followed by malleus than incus (Sodhi et al., 2017).

A Fetal cadaveric study was done which used a conventional method that comprised of 15 adult and 22 fetal cadavers. A mortuary in the Anatomy department of Ajman (UAE) provided adult while the fetal ones were granted by Gynecology department. By entering through tegmen tympani the tympanic cavity was approached to obtain these ossicles. Screw gauge with a (least count of 0.05) was taken into consideration for measuring the dimensions. A microscope (stereoscopic) was used to take the photographs according to its proper guidelines available (Nadeem, 2013).

A study on primates used extracted ossicles from the temporal bone and then a CT scan was taken afterwards with resolution in the range of (0.010-0.020 mm). If there was a chance of distortion of these bones during the extraction procedure, it was than avoided and a complete scan of the skull with quality resolution was taken (0.022-0.091 mm). The sample of these bones were provided from Anatomy department of German university (Leipzig). This investigation presented a mathematical morphometric estimation convention for 3D shape examination dependent on milestones and semi landmarks acquired from mCT pictures and apply it to ossicles of surviving primates (extraordinary chimps and people). It showed that the convention was dependable and reproducible over a scope of voxel goals, and caught even unpretentious shape contrasts. Utilizing this methodology it is feasible to recognize the primate taxa by mean states of their malleus and incus ($p < 0.01$). At last, the consequences of this examination gave a relative system to morphometric considers breaking down ossicles of terminated primates, with a course on scientific classification, phylogeny and hear-able capacity (Stoessel et al., 2015).

The point of this investigation was to track down the morphological varieties of ear ossicles of right and left sides. In this investigation, the ear ossicles were respectively analyzed out from the transient bones contracted from 26 cadaveric heads from the Department of Anatomy under a careful oto-magnifying lens with miniature instruments.

Morphological varieties were concentrated under the amplification of the working magnifying instrument. Endeavor was likewise made to assess the clinical ramifications identified with such variations and in different places of India and abroad comparison were made. Age variety was not considered in this investigation as it considered this school of thought that the ossicles at birth arrive at their full size. After ethical approval the extraction procedure of the bones were carried with the common technique called (intact canal wall mastoidectomy-inside out) (Lucidi et al., 2019).

The roof top needs to be drilled and opened up in order to expose (malleus head, body and short process of incus). Epitympanum lateral wall was removed initially to do so. The attachment of these bones to the ear drum is achieved by the help of ligaments, so they should be removed for easy extraction of these bones. It begins with the separation of malleus from incus body. After that division of joint between incus and stapes (incudo-stapedial) was done to visualize stapes dorso-medially. Variations amongst these bones were identified using magnified operating microscope. Parameters of morphology were compared between left and right ear ossicles with application of chi-square test ($p < 0.05$) taken significant (Saha et al., 2017)

The malleus is the biggest bone amongst other ossicles. In the current investigation, it attempted to decide the typical scope of different estimations of malleus and whether these estimations are helpful for the sexual dimorphism or not. Out of absolute 60 malleus utilized in the current examination, 30 were recovered from the male dead bodies and remaining from the female corpses. Mean of absolute length of malleus, its manubrium and head and neck in male are more when matched with female in the current examination. Amongst all estimations taken in the current examination; complete length of malleus having measurably huge distinction between the male and female malleus and it tends to be utilized for the sexual dimorphism of malleus for population Kachchhi Gujarati of present investigation. Following estimations of the malleus were taken by utilizing computerized Vernier caliper. To reduce the intra-observer variation, every estimation was taken at three unique occasions and the mean of each of the three readings was taken as the last perusing (Javia & Saravanan, 2020).

The otologic specialists should be completely acquainted with the anatomical subtleties of center ear and its bones in order to perform microsurgical moves effectively. In India, information on typical anatomical boundaries of the ossicles is restricted. The current examination endeavors to give the anatomical subtleties of the three ossicles in North Indian Population and match the boundaries with those announced from the various areas of the world. The examination was completed on 100 arrangements of center ear ossicles gathered from grown-up male dead bodies from the Department of Anatomy of different universities of North India. The different estimations were taken with the assistance of advanced Vernier caliper. This study uncovers no huge distinction between morphometric estimations of the bones of both side of the ear, except male's incus (Sodhi et al., 2017).

This examination explores the morphometric and morphologic varieties of center ear ossicles, taken from 40 infant bodies of both genders which were analyzed reciprocally. This investigation was performed on dead bodies from the University of Erciyes in Laboratory of Anatomy. The ossicles were acquired from tympanic cavity subsequent to opening tegmen tympani. The estimations were assessed with a micrometer named as Mitutoyo which has an exactness of (0.01 mm). A stereoscopic magnifying instrument was used for taking pictures. The estimations of angles were assessed on the photos. The incus had least morphological varieties in the ossicles. Stapes had most extreme morphological varieties among all ear ossicle. It was seen that while a few ossicles were very much grown morphometrically, their bone mass didn't arrive at an adequate size. (Doğanlarda et al., 2002)

A high resolution image of middle ear was taken with the help of a high frequency ultrasound investigation. It helped in visualizing the middle ear structures that included (TM, unfixed cadaver ears, ossicles, and its supporting tissues) which were easily detectable. It was capable enough to reveal the structures present in the center part of the ear by providing high quality image with real time ultrasound, its vibrometry and captured quality videos. Its high end results concluded that this method is very simple and less invasive technique as compare to other investigation tools. This brilliant technology helped clinicians to understand and diagnose the ear pathologies mainly regarding the middle part with the extent of improving the quality of life. In order to create images of middle ear as mentioned, first time world's commercially accessible scanner (HFUS linear array) was used. An imaging system (Vevo 2100, VisualSonics, Canada) was used to perform Ultrasonography (Figure 2.5 and 2.6) (Lindsey et al., 2017).

Partial submersion of bones in a bath containing liquid saline with vertical placement of TM and cavity of middle ear was opened to allow this fluid to flow freely in it. A gel was applied on the exposed area to contain the fluid (Figure 2.4). Several transducer positions were acquired to capture required images of middle ear to visualize best anatomical views of interest (Landry et al., 2015).

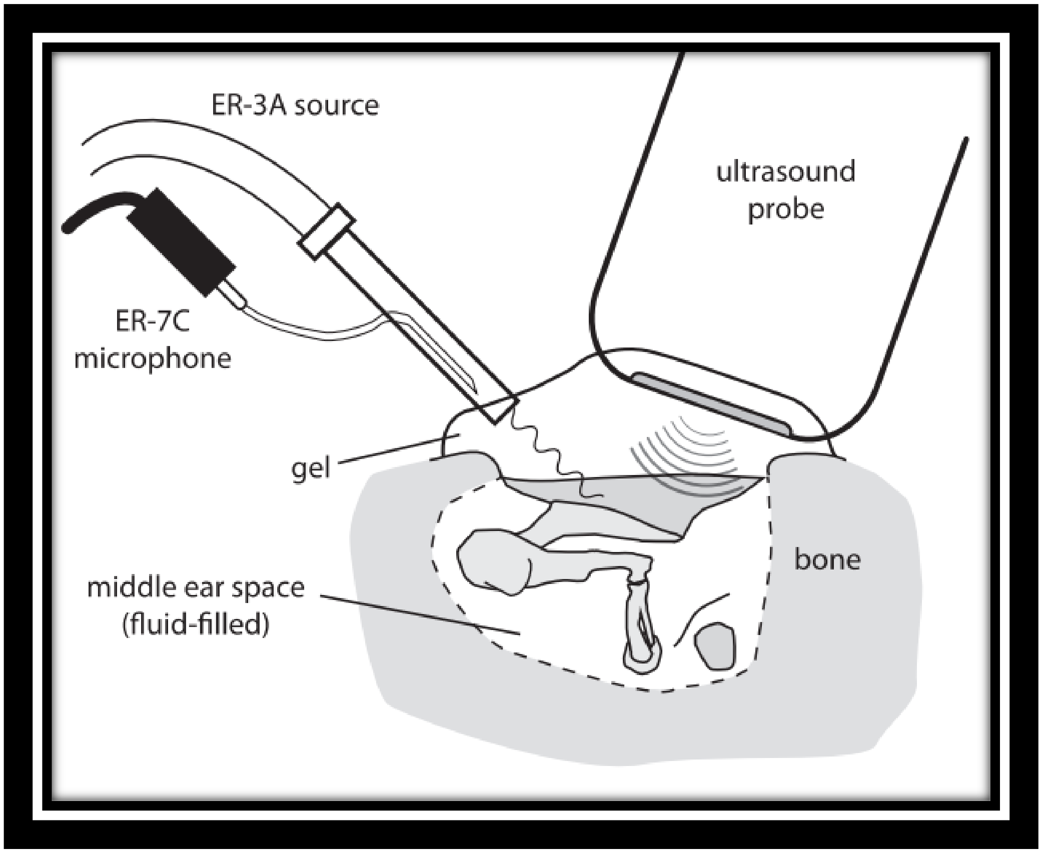


Figure 2.4 A schematic diagrammatic representation of imaging probe arrangement, sound presentation and measurement devices during middle ear vibrometry (Landry et al., 2015).

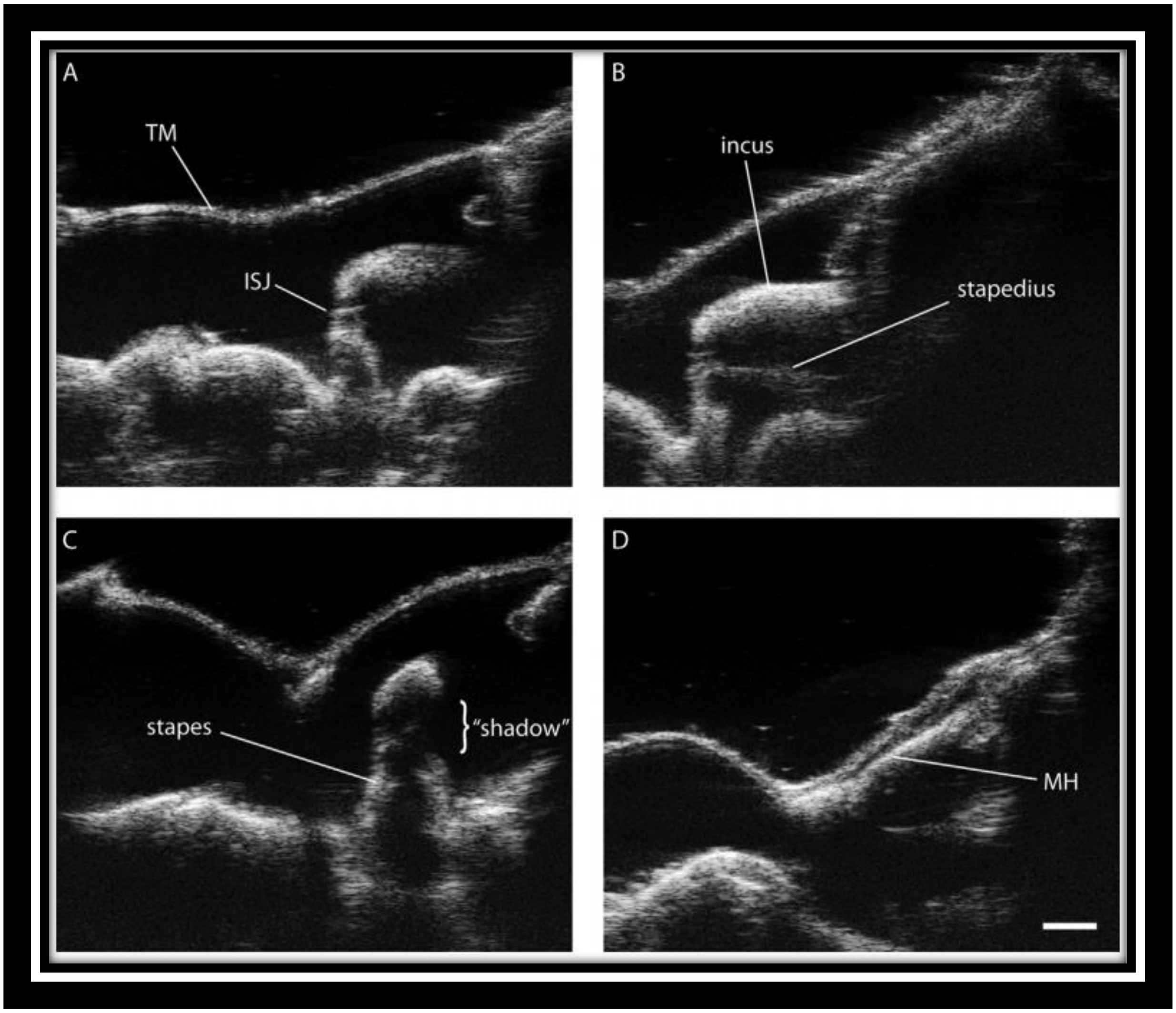


Figure 2.5 HFUS images of middle ear in a cadaveric tissue sample from an 89 year old female. TM is clearly visible. Incus, Incudostapedial joint (ISJ), stapedius muscle and malleus handle (MH) can be appreciated (Landry et al., 2015).

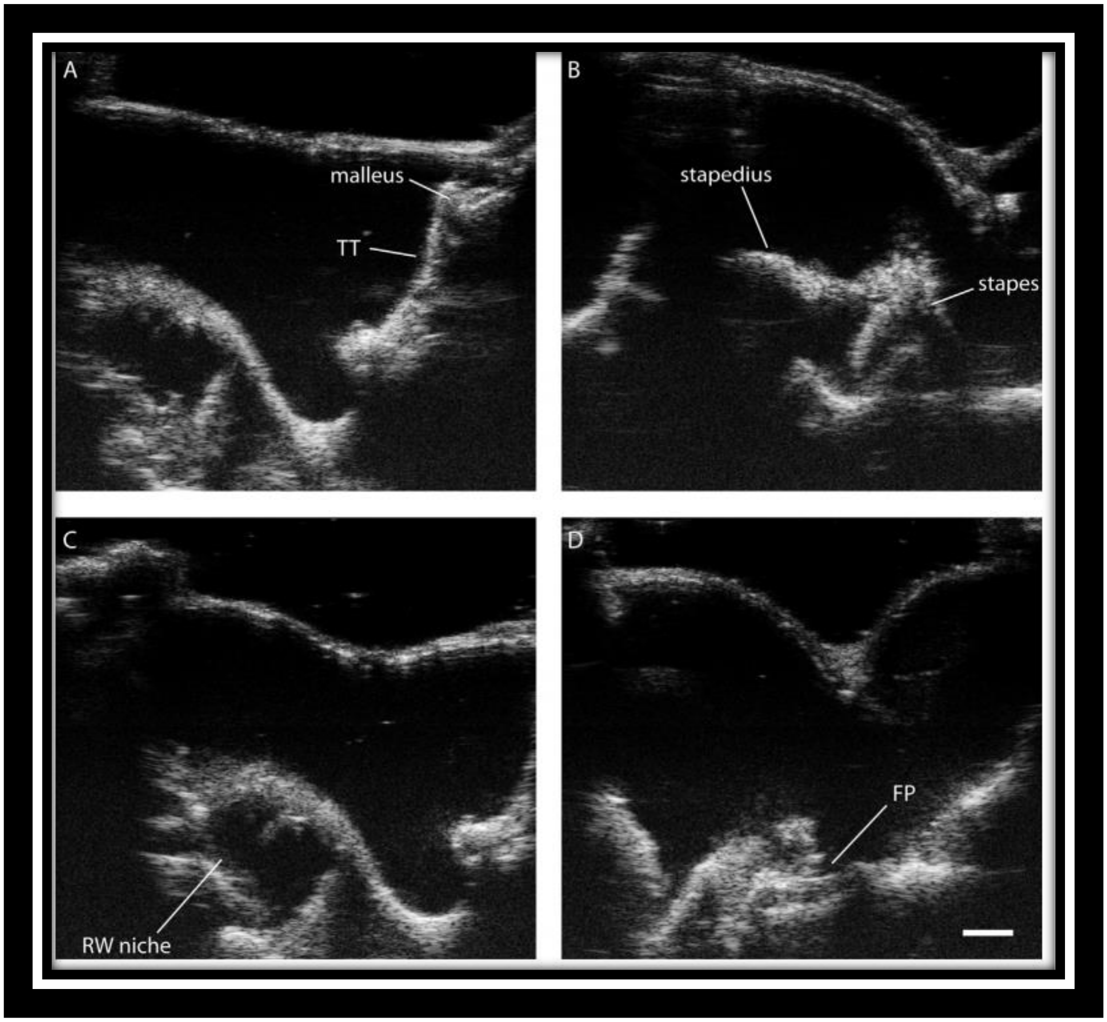


Figure 2.6: HFUS images of middle ear of cadaveric sample from a 48 year old male. Highlighted here is malleus, stapedius muscle, tensor tympani muscle (TT), stapes crura, Round window niche (RW) and stapes footplate (FP) (Landry et al., 2015).

This is a study which included retrospective data, it compared the morphological variation seen in ear ossicles of normal subjects (20n) with congenital aural stenosis (20n) and atresia (20n) patients. A CT scan imaging technique was adhere to detect these changes in digital. All images were obtained using a standard temporal bone protocol. Slice thickness of CT film taken in consideration was of 0.75 mm. The 2-D (DICOM) collected data were entered into a software (Mimics 12.1). A diagrammatic representation of the ossicles has been provided in (Figure: 2.7) which shows the landmarks which were taken into account while noting the morphological variations in millimeter scale (mm) of these ossicles. A 3-D schematic reconstruction established landmarks, were taken as criteria for measurement of these ossicles on CT scan (Figure 2.8). In order to precisely define the places of the head of malleus and a joint (incudomalloelar joint), a standard 3-D facilitate framework was utilized in light of a plane named as Frankfurt horizontal (Pfrkt) and 2 perpendicular planes (Li et al., 2016) OssicleCalculation is an application which was used to enter all landmarks with 3-D coordinates inscribed in Matlab (Mathworks, Cambridge, UK) to calculate the angles between lines, point-to-plane and point-to-point distances.

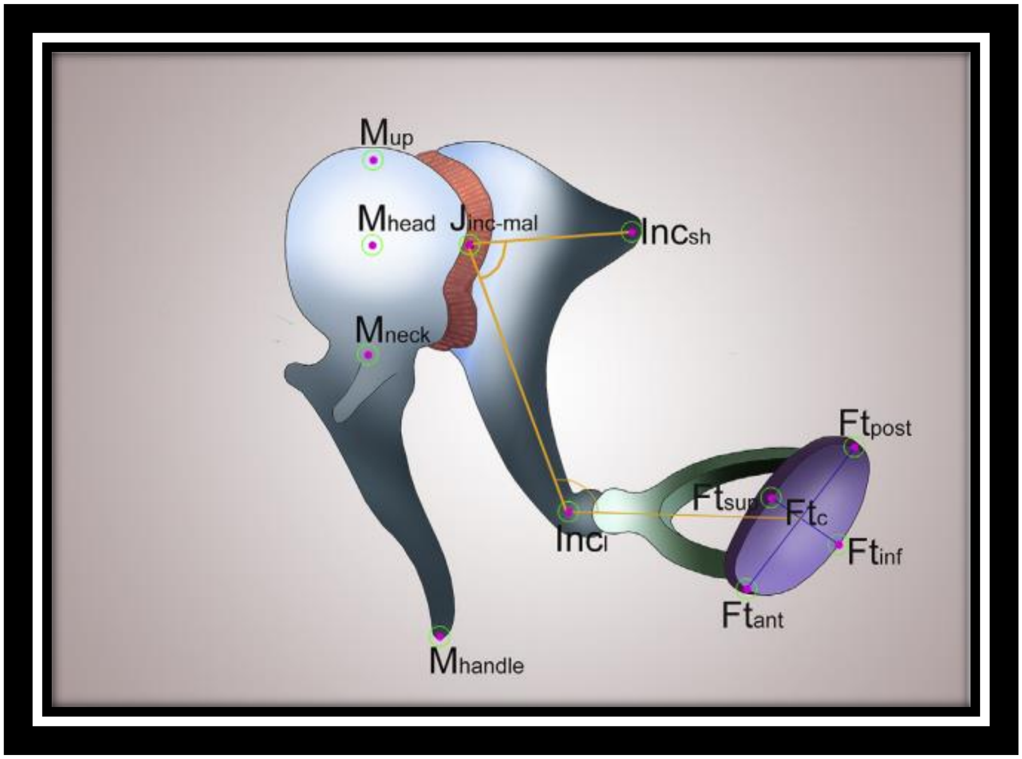


Figure 2.7 Landmarks for measurements: The central points of the malleus head (Mhead), the malleus neck (Mneck), and the incudomalloelar joint (Jinc-mal); the distal ends of the long process of the incus (Incl), the short process of the incus (Incsh), and the malleus handle (Mhandle); the upper point of the malleus head (Mup); the anterior point of the stapes footplate (Ftant); the posterior point of the stapes footplate (Ftpost); the midpoint of the superior border of the stapes footplate (Ftsup); the midpoint of the inferior borders of the stapes footplate (Ftinf); and the central point of the stapes footplate (Ftc) (Li et al., 2017).

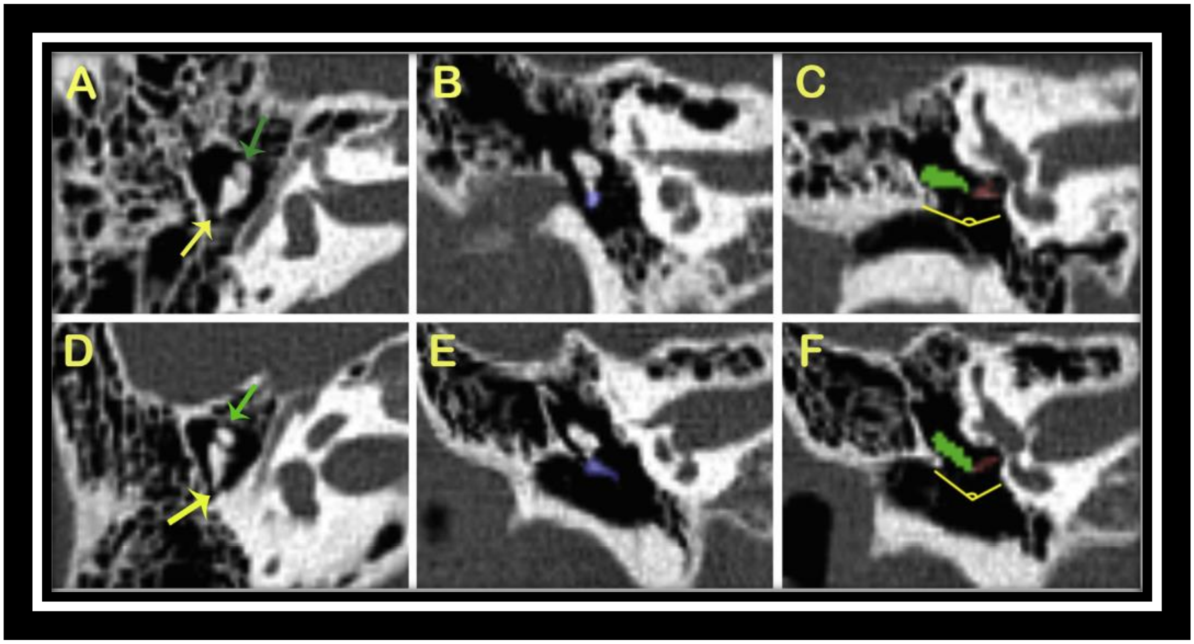


Figure 2.8 Representative HRCT images of the ossicular chains in ears in with (CAS; A-C) and normal ears (D-F). (A and D) The size of the malleus head (green arrows) and the length of the short process of the incus (yellow arrows). (B and E) The length of the malleus handle (blue). (C and F) The length of the long process of the incus (green) and the angle of the incudostapedial joint (yellow). The red bones in C and F are stapes (Li et al., 2017).

CHAPTER 3

METHODOLOGY

3.1 Study design

Descriptive cross-sectional study design has been implemented in the current work. This research is a human based study and was done in the period of six months January-June 2021. This work was conducted at ENT and Radiology Departments of PNS Shifa Hospital Karachi. Initially ethical approval from Ethical Review Committee (ERC) of Bahria University of Medical and Dental College (BUMDC) for this project was obtained. The subjects who visited the ENT department were referred to Radiology Department for radiological investigation and were considered in this research after fulfilling the inclusive criteria and written informed consent.

3.2 Subjects

Human subjects with intact ear ossicles were selected and arranged in five age groups.

3.3 Setting

ENT and radiology Department of PNS Shifa, Karachi.

3.4 Inclusion criteria

- Both genders
- Subjects were divided into five age groups: A (10-20), B (21-30), C (31-40), D (41-50), and E (51- onwards) years old.
- Subjects with intact ear ossicles of one side of the ear

3.5 Exclusion criteria

- Fractures involving ear were excluded
- Poly trauma patients
- Participants who underwent any form of surgery of the ear in the past related to ear ossicles
- The eroded ossicles

3.6 Duration of study

a) Individual study period: 2-3 hours per case
b) Total period of study: 6 months

3.7 Sample size estimation

The present study "Morphological variations in ear ossicles with age in correlation with hearing thresholds", sample size was estimated using method of sample size for frequency in a population www.openepi.com which is an open source calculator, version 3-SSPropor using following equation;

Sample size $n = [DEFF*Np(1-p)]/[(d^2/Z^2_{1-\alpha}*(N-1)+p*(1p)]$

3.8 Sampling technique

Non-Probability convenience sampling technique was applied for this research project, all the subjects were selected non-randomly. The samples were collected for specific purpose with predetermined basis of selection which was based on inclusion and exclusion criteria of the study.

3.9 Human subjects and consent

The consent forms for subjects were designed in both English and Urdu which included all the information about the research work. The written informed consent was obtained from all subjects. The consent forms are attached as Appendix C and D.

3.10 MATERIALS used (Drugs/ Chemical/ Proforma/ Questionnaire and any other)

The materials used to conduct this study include consent forms in English and Urdu, CT scan machine (Prime Aquilion-160 slice Toshiba) (Figure 3.1), CT reporting room (Figure 3.2), CT scan reports and Questionnaire.

The instruments used for ear examination include Otoscope (Figure 3.3), ENT unit (suction, spray) (Figure 3.4), Examination instruments (ear) (Figure 3.5), Tuning fork (Figure 3.6), Ear speculum (Figure 3.7), Jobson Horne probe (Figure 3.8), Head mounted lights with head band (Figure 3.9), Suction (Figure 3.10), Tilly's aural dressing forceps (Figure 3.11) and Pure Tone Audiometer (Figure 3.12).

Figure 3.1 CT scan machine (Prime Aquilion-160 slice Toshiba) shows patient undergoing HRCT of petrous temporal bone at PNS Shifa Hospital Karachi.

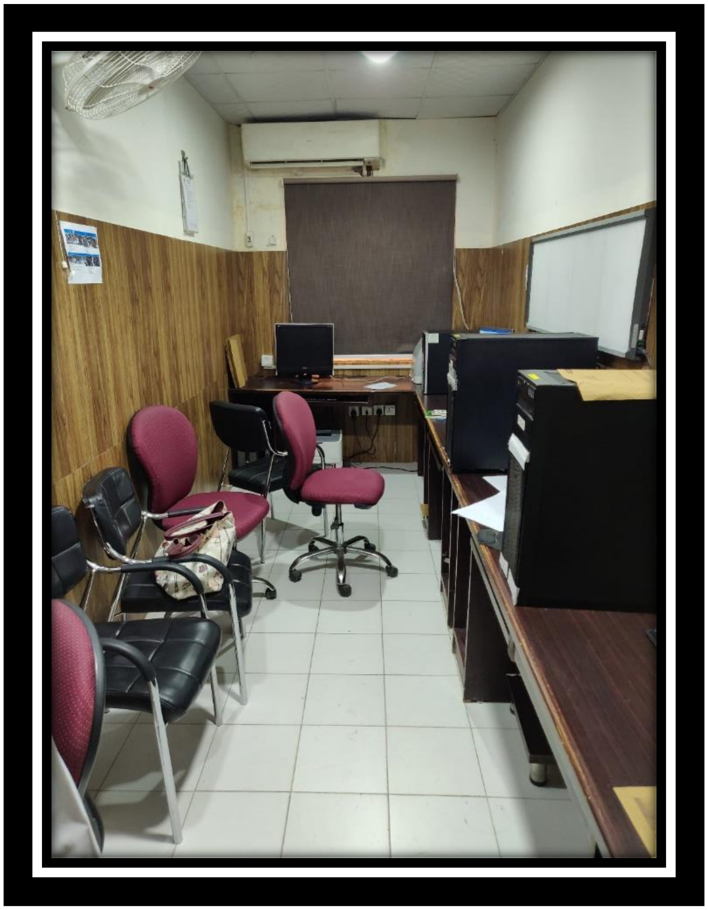


Figure 3.2 CT scan reporting room (PNS-Shifa Hospital Karachi)

Figure 3.3 Otoscope to examine external auditory meatus and ear drum

Figure 3.4 ENT unit (suction, spray)

Figure 3.5 Ear examination instruments

Figure 3.6 Tuning fork for clinical tests of hearing loss; vibration test

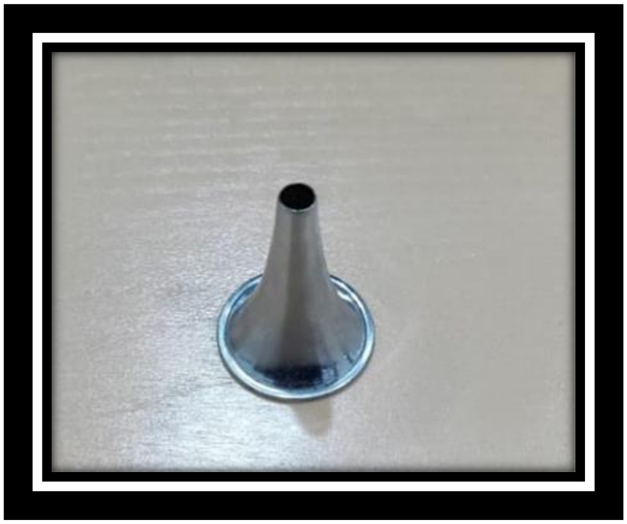


Figure 3.7 Ear speculum for ear examination

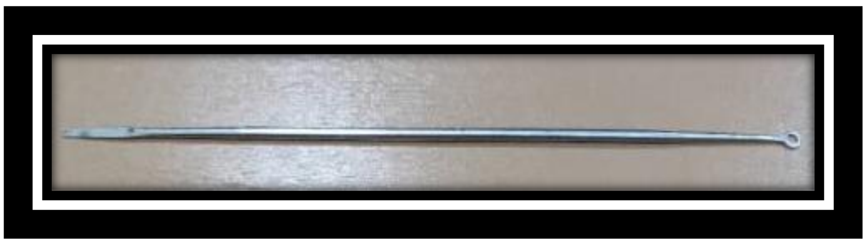


Figure 3.8 Jobson Horne probe used to remove any foreign body from the ear

Figure 3.9 Head light used to focus light into cavity under inspection

Figure 3.10 Suction that helps to remove ear wax and allows visibility of ear drum

Figure 3.11 Tilly’s Forceps is used to remove foreign particles from the ear

Figure 3.12 Pure Tone Audiometer for recording hearing thresholds (dB)

3.11 Parameters of the study

- Variations on the basis of:
    - Malleus and incus
    - Different age groups
    - Gender
    - Ethnic group
    - Side of the ear
    - Shape of manubrium (curved or straight)
    - Measurements of malleus (head width, length of manubrium and total length)
    - Measurements of incus (width of short process & long process)
    - Hearing thresholds

3.12 Protocol of study

Patient fulfilling inclusion criteria were called after taking detailed history and examination, after taking written and verbal informed consent form. History comprised of details of (demographic data including name, Age, height, weight, ethnicity, marital status, job description, education), presenting complain, family history, history of comorbities, side of the ear involved, assessment of hearing, clinical history, surgical history, history of trauma, workplace, clinical investigation, name of investigations performed.

3.12.1 Examination protocol

In order to check that ear is totally fine and it meets the inclusion criteria, a thorough ear examination was done and with the help of instruments utilized for routine assessment of ear such as otoscope, frenzel's glasses, head reflect, tuning forks and for evaluating the hearing misfortune Pure tone audiometer (Liaw, Patel & Carr, 2017).

First and foremost pinna and the encompassing skin was surveyed for any anomaly. Search likewise for pre-auricular pits and sinuses. If the external meatus is clear, the scalp should also be examined as a site of primary infection. (Glynn et al., 2017)

3.12.1.1 External auditory canal

A hand-held auriscope with a head reflect was used to inspect canal, utilizing mirrored light and an aural speculum fitted with an amplifying focal point (Siegel's speculum). To accomplish a decent view cartilaginous meatus was align with the hard meatus, in a young participants this was best accomplished by withdrawing the pinna in reverse and in grown-up by withdrawing the pinna upwards and in reverse. An auriscope ought to be held was like a pen among thumb and forefinger with ulnar order of the hand leaning delicately against the sides of the participants head. In this manner any movement of head during assessment was obliged by proper handling of auriscope, restricting any danger of direct injury to the ear channel if the participant moves surprisingly.

After assessment of outer ear canal, the tympanic film was assessed. The entire film was encircled by a sinewy ring (annulus) which fixes the drum strongly to the encompassing bone (Pedler & Chang, 2005).

3.12.2 Assessment of hearing

During clinical testing, because of the intervention of the mass of head, sounds were weakened or veiled in the ear farthest from the sound source by a sum that changes as per the recurrence of the sound and the closeness of the sound to the test ear. Along these lines a vibrating tuning fork was held close to the test ear, for reasonable designs, to be heard distinctly in that ear. Notwithstanding, when a similar tuning fork was set on the mastoid interaction of the test ear, it was heard to be at a similar power in the non-test ear given that the two ears were having same ordinary hearing (Shapiro, 2011).

Hearing was assessed by asking the patient whether he/she can hear the doorbell or phone (sound yields around 60dB) and inquire as to whether discussion in a peaceful climate can be heard (ordinary levels around 40 dB) (Leblanc, 2013).

Clinical testing was also be performed by asking the patient to repeat words spoken at varying intensities. Such free-field voice testing was done by using phonetically balanced words (e.g. dustbin), number combinations (e.g. ‘6-3-4’) or combination of numbers and letters (e.g. ‘7-M-5’). For these tests the examiner stood up to the side of the ear to be tested and masked the non-test ear using tragal movement. The test started with a whispered voice at 60cm (2 feet) (approximated intensity 15 dB) and proceeded with a whispered voice at 15cm (6 inches) (intensity about 35 dB). When there was no response a conventional voice at 60cm (2 feet) (50 dB) was used and this was then repeated, when it was necessary, at 15cm (6 inches) (55-60 dB) from the test ear. However, responses in this simple test were difficult to quantify in some participants with poor English comprehension. Formal audiometry, therefore, was usually necessary (Gardner, Hickmott & Ludvik, 2012).

Further information about hearing was obtained at the bedside by using a vibrating tuning fork. For these tests tuning forks of 512 Hz were used since higher frequencies are less accurate at identifying differences between air and bone conduction, and lower frequency forks produce vibrations which may be misinterpreted by the participants as sound. Tuning forks as heavy as possible were used as with these the sound levels decayed less rapidly (Glynn & Drake, 2017).

In clinical practice two test were employed. The Rinne test which helps in comparing the hearing by air and bone conduction. It was performed by striking the tuning fork and holding it in line with the external auditory canal (air conduction) and then against the post auricular skin (bone conduction). The participant was asked in which test position the sound was heard louder. In normal subjects the sound was better in air conduction than bone conduction (Rinne positive) and this response is also seen in sensorineural deafness. In conductive deafness the converse was true (Rinne negative) (Gupta et al., 2018).

In Weber test the base of vibrating tuning fork was placed anywhere on the midline of the skull and the participant was asked whether the sound was heard in the midline or it was lateralized. The normal response was to localize the sound to the midline; this was also true when the hearing was symmetrically reduced. However, when there was normal hearing on one side and a pure sensorineural loss on the other, tuning fork was heard to be louder in the normal ear. Conversely, when there was a purely conductive hearing loss in one ear and normal hearing in the other the tuning fork was heard louder on the side with conductive defect. Two fallacies exist with Rinne test. The first is that up to one-third of patients with an air-borne gap of 30 dB with Rinne positive and it is only when the difference between air and bone conduction exceeds 40 dB that the Rinne test is negative on 90% of occasions. This lack of specificity is often important clinically. Secondly patients with a profound unilateral sensorineural hearing loss or a dead ear will report a Rinne-negative response when this ear is tested if the better ear has normal or reasonable hearing. This is because of the lack of attenuation of bone-conducted sound which is heard in the non-test ear. This problem is alluded to above and, for this reason, a Barany box should be used to mask the non-test ear when a negative response is obtained (Ungar et al., 2019).

After this thorough examination it was seen whether the participants met the inclusive and exclusive criteria. Radiological results were interpreted according to the parameters. The data collection was completed from January to April 2021. Analysis of data and result compilation was performed in a month, followed by thesis completion. The total duration of study was 8 months.

3.12.3 Protocol for HRCT PTB

After ear examination and evaluation of the inclusion and exclusion criteria. Each individual was referred for CT-scan of the petrous temporal bone having unilateral disease of the ear to the department of radiology PNS Shifa. Such individuals were told about the study and were asked to sign an informed consent form. The risks and benefits of the study were told and it was made sure that they understood the procedure. The scans were taken by same CT machine. They were then evaluated for the measurement of ear ossicles by the principle investigator, and were cross checked by an expert radiologist. Since the CT-scan was of the entire head region, petrous temporal bone and the ear ossicles of both sides were observed. Measurements of ear ossicles of only the normal ear were recorded in three different planes; coronal, sagittal, and axial. The diseased ear was not considered and its measurements were not recorded for this study.

3.12.4 Positioning of the patient

HRCT is a type of CT scan with specific techniques to enhance image resolution. For this study the Aquilion-160 slice Toshiba Prime CT scanner was utilized. This series of scanner merges unparalleled flexibility and cutting edge technology enabling patients of all ages and sizes to be handled. It employs a sophisticated reconstruction method to decrease metallic artifact, improving visualization of implants, supporting bone and the adjacent soft tissues. The individual was placed in a fully supine and still position on the 47 cm wide couch. The couch was positioned to place the lens of the individual's eyes as far as possible out of the pathway of the x-ray beam to minimize exposure to the lens. The bore of the CT scanner was 78 cm in width. It produced images with a 0.5 mm resolution and 40% better light output. With respect to goal, the thickness of each cut was 0.75 mm with a 0.5 mm increase for each cut, a 512 × 512 lattice, also, pixel size of 0.43 mm. The showcase field-of-perspective on each cut was 22.0 × 22.0 cm.

3.12.5 CT scan image evaluation:

Measurements of ear ossicles were taken in the console room for CT with the help of a software (Vitrea 1.5.2265.3045). It can be seen in (Figures 3.13 to 3.18) that how the steps were followed for recording of changes observed in the morphology of ear ossicles.

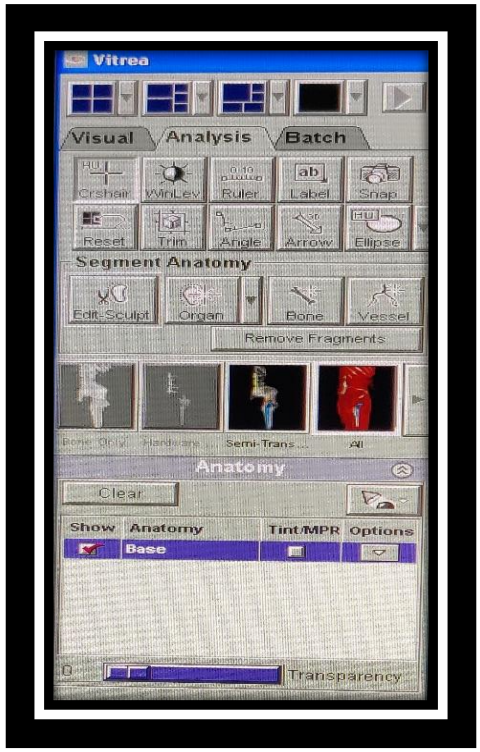


Figure 3.13 Vitrea software (5.1.2265.3045) having ruler to measure ear ossicles in millimeter (mm)

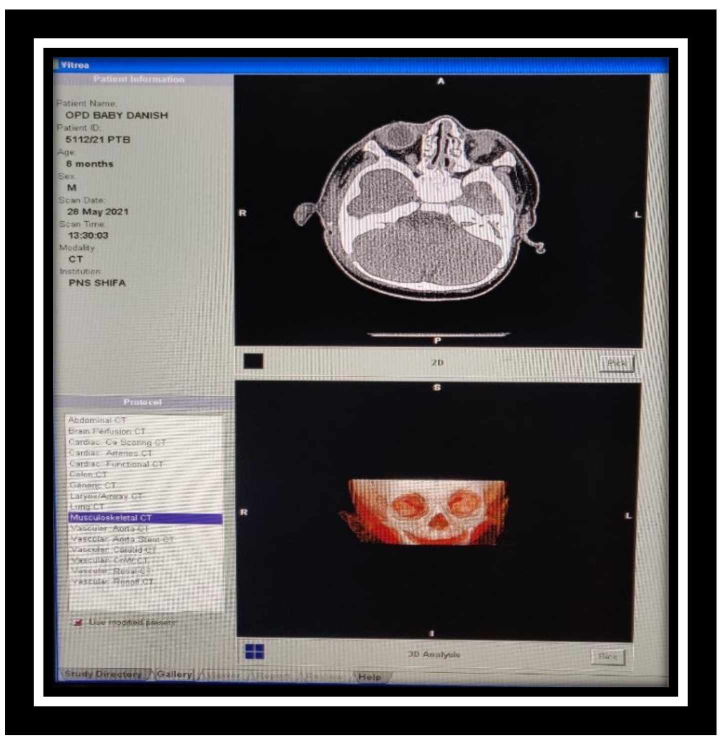


Figure 3.14 Vitrea software appreciating Musculoskeletal CT allowing both 2 & 3-D analysis

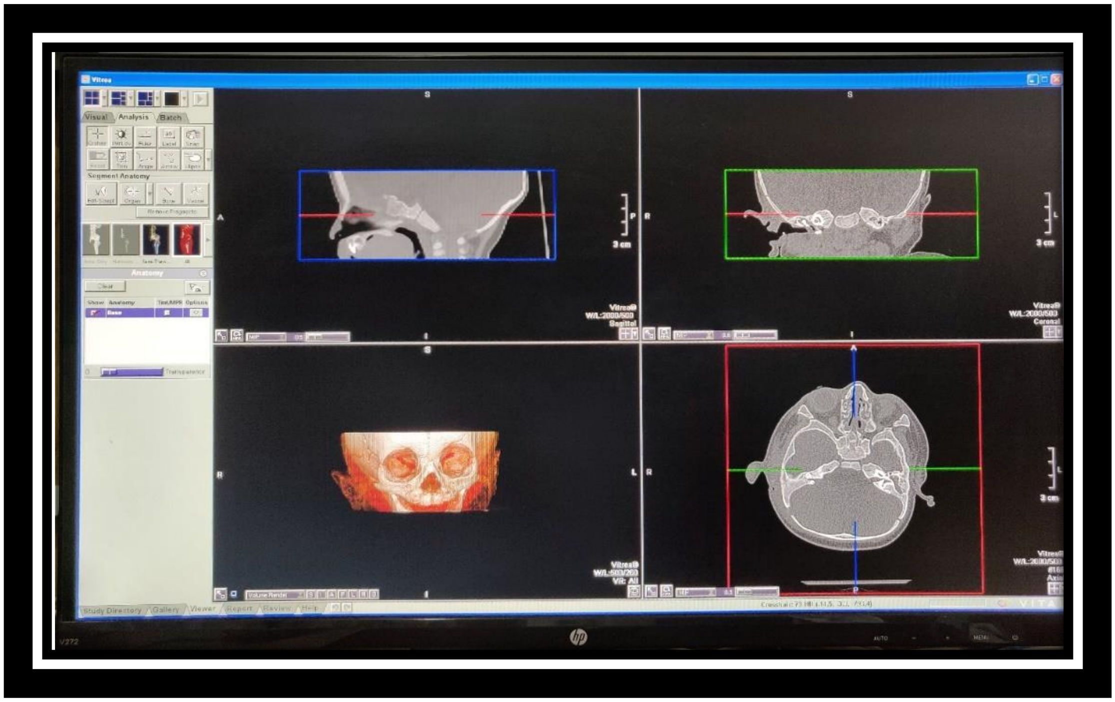


Figure 3.15 (Sagittal, Coronal and Axial views)

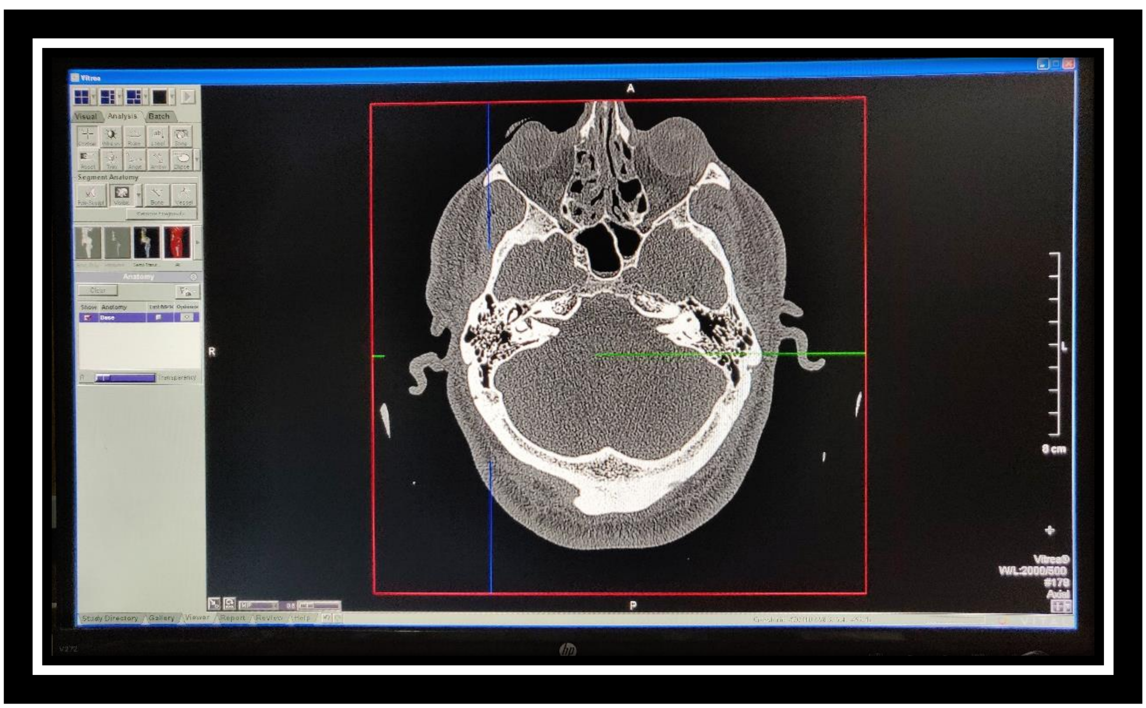


Figure 3.16 Axial view showing Malleus head and body of incus

Figure 3.17 Coronal view showing Malleus head, neck and lateral process

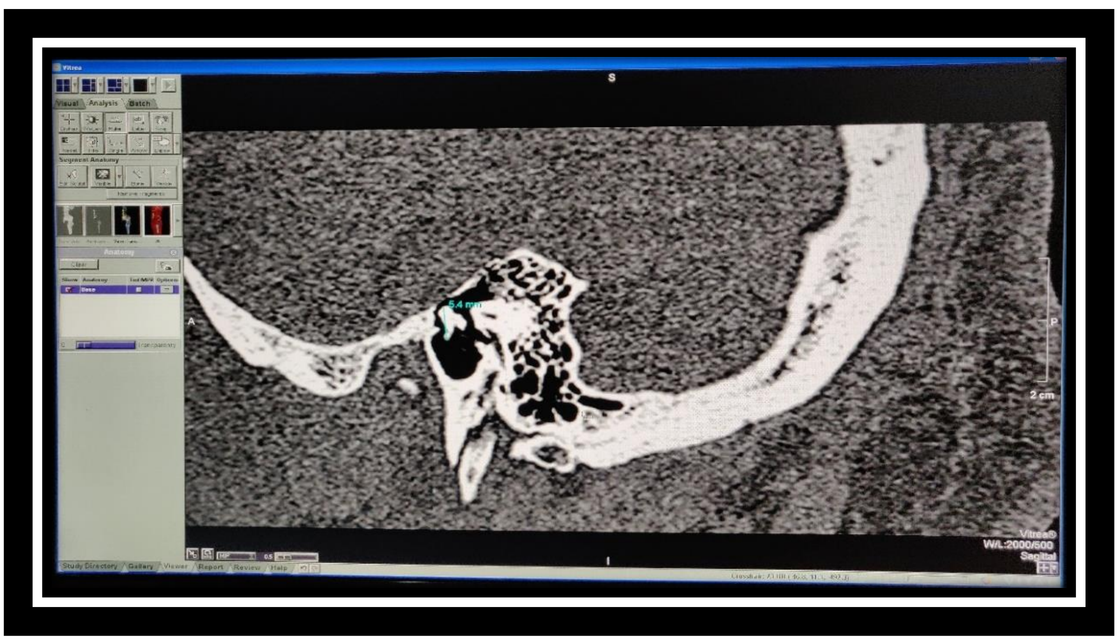


Figure 3.18 Sagittal view showing Malleus and Incus

3.12.6 Protocol followed for taking measurements:

Landmarks that were considered as a reference criteria for measurements were the malleus upper part (Mup), central point of the malleus head (Mhead) and the malleus neck (Mneck) for measuring width of malleus. For measurement of length of malleus handle, the malleus neck (Mneck) till malleus handle (Mhandle) was taken in consideration. Malleus upper point (Mup) and malleus handle (Mhandle) for measuring total length of malleus. The length of short process of incus was measured by taking measurement starting from junction between malleus and incus (Jinc-mal) till end point of incus short process (InCsh). The total length of incus was measured from upper point of incus till end point of long process of incus (InCi). The points taken into account are shown in (Figure 3.19) (Li et al., 2017).

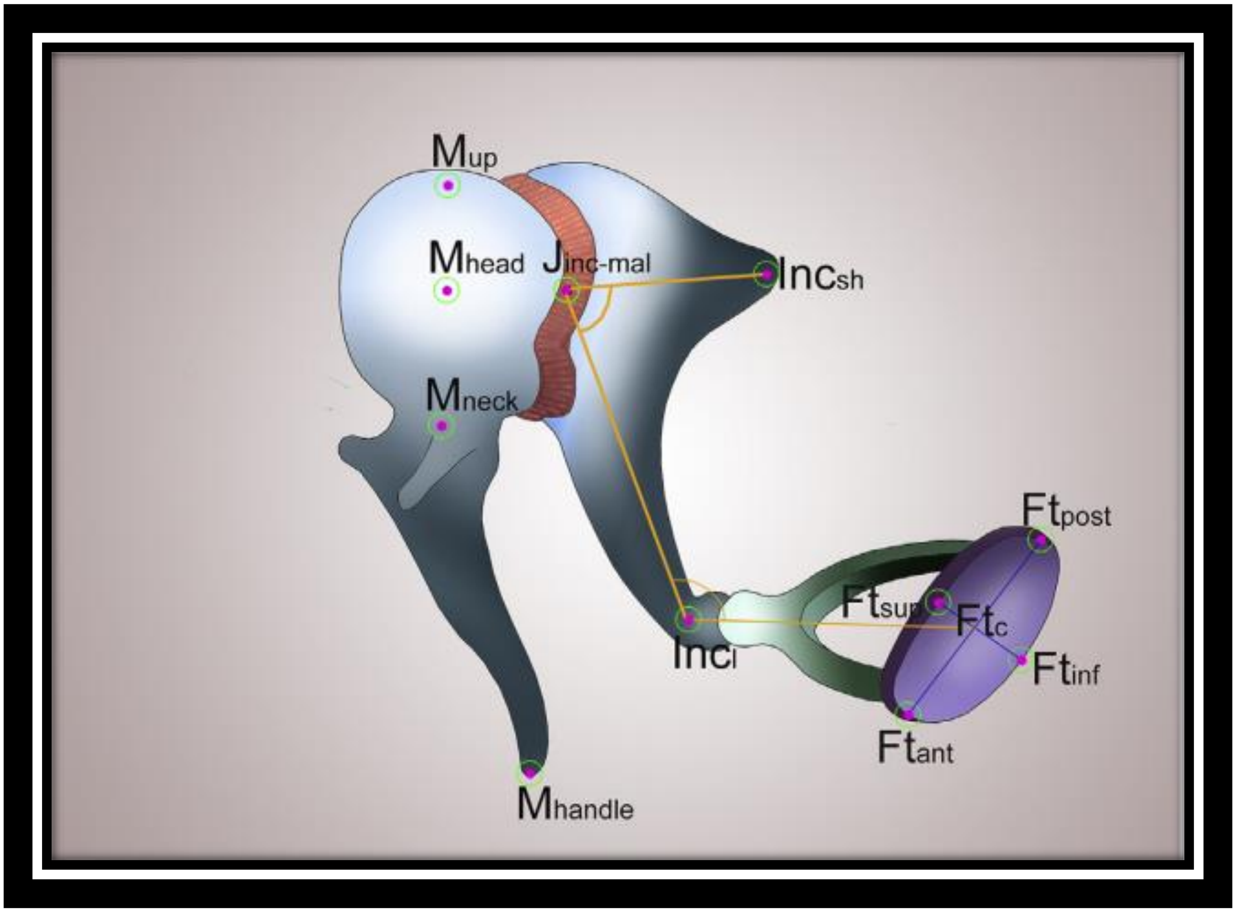
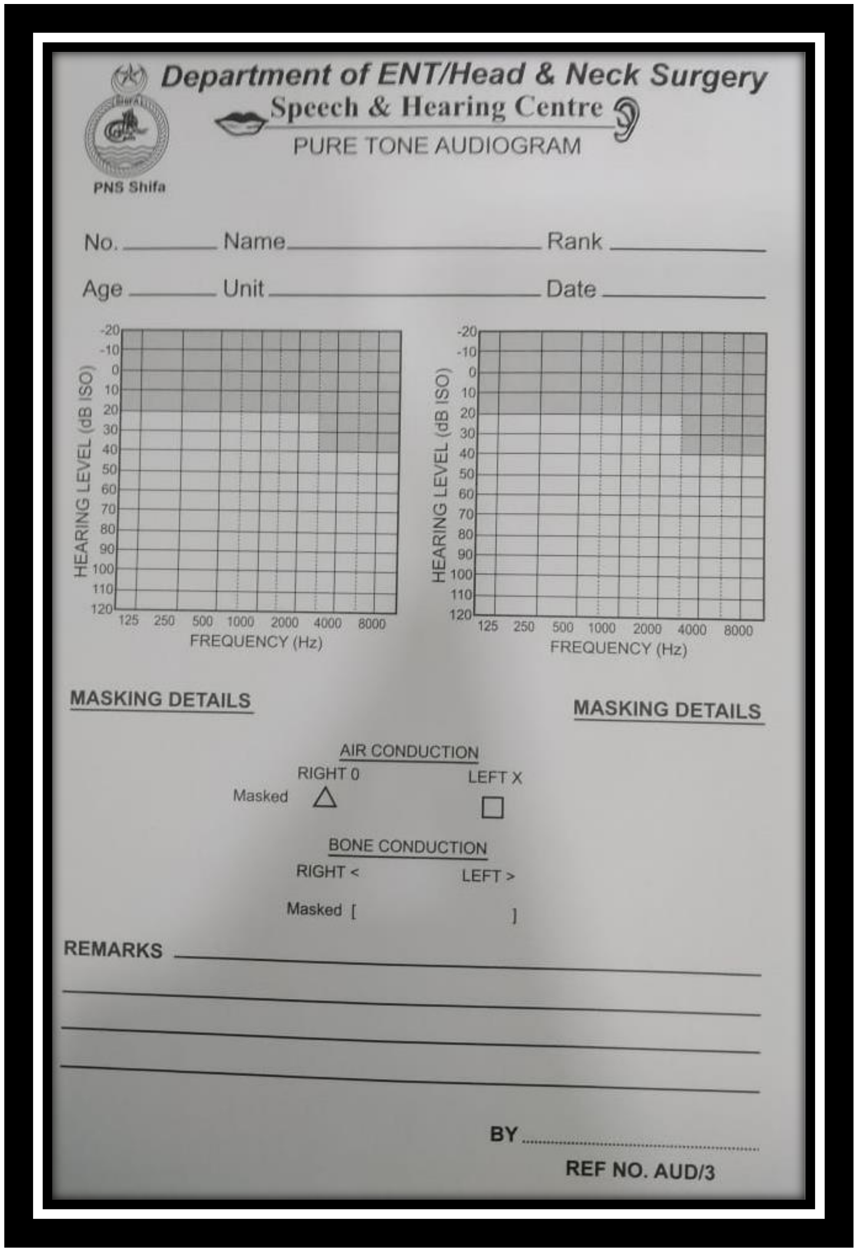


Figure 3.19 Landmarks considered for taking measurements (Li et al., 2017)

3.12.7 Protocol for audiometry test

A hearing test is performed on all the participants to evaluate the hearing threshold levels in all the individuals. The variations were noted amongst all the six age groups and correlated with the ear ossicles. The readings were noted on audiogram card (Figure 3.20).



Department of ENT/Head & Neck Surgery
Speech & Hearing Centre
PURE TONE AUDIOGRAM
PNS Shifa

No. ______ Name ______________ Rank ______

Age ______ Unit ______________ Date ______

HEARING LEVEL (dB ISO) -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
FREQUENCY (Hz) 125 250 500 1000 2000 4000 8000

HEARING LEVEL (dB ISO) -20 -10 0 10 20 30 40 50 60 70 80 90 100 110 120
FREQUENCY (Hz) 125 250 500 1000 2000 4000 8000

MASKING DETAILS

MASKING DETAILS

AIR CONDUCTION
RIGHT 0 LEFT X
Masked △ □

BONE CONDUCTION
RIGHT < LEFT >
Masked [ ]

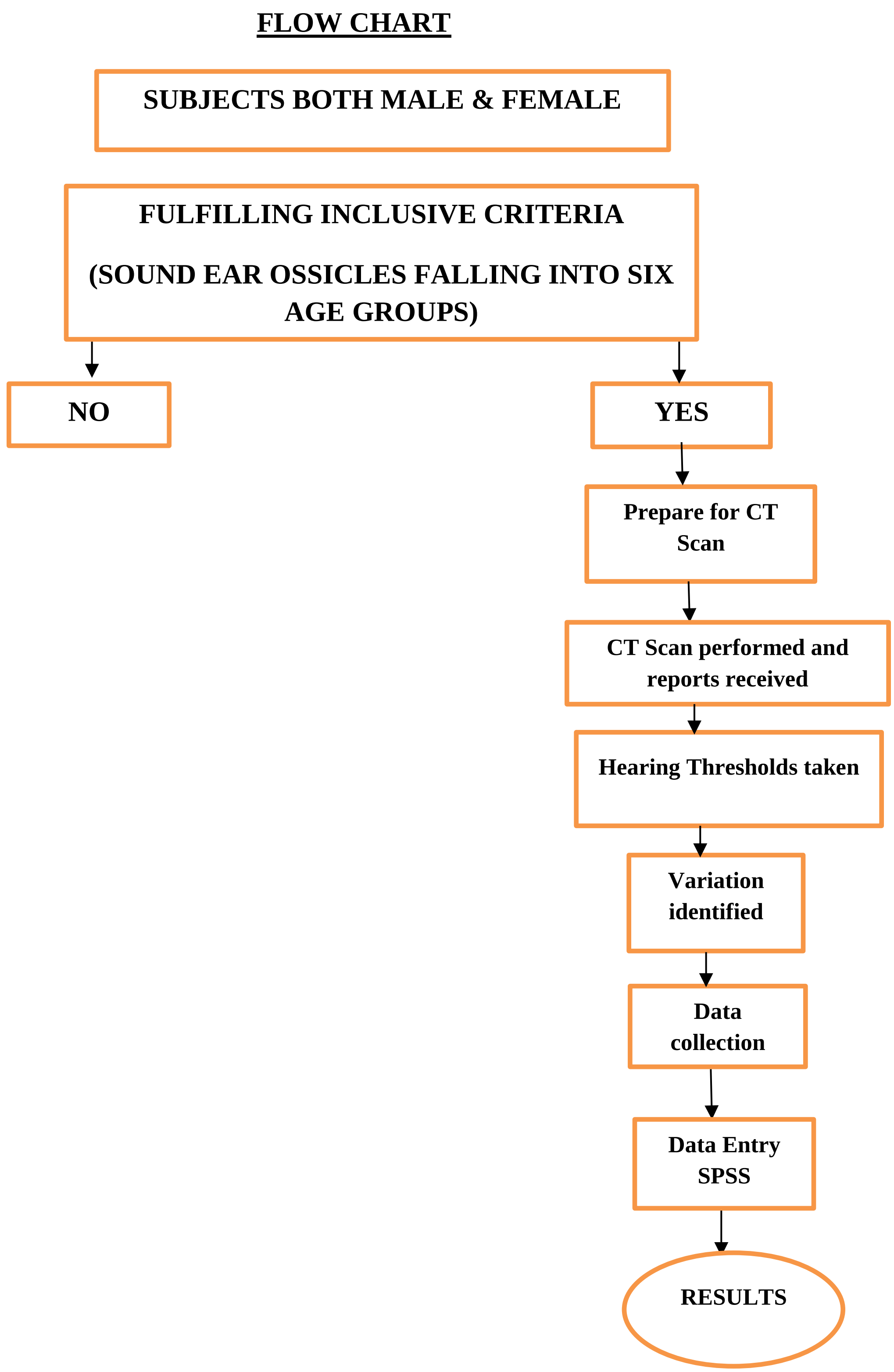
REMARKS ______________________

BY ....

REF NO. AUD/3

Figure 3.20 An audiogram card for noting hearing levels (dB)

3.13 Algorithm of study



3.14 Statistical analysis

Statistical analysis was done using the Statistical Package for Social Science (SPSS) software version 23.0. All continuous variables were presented as mean ± Standard deviation. All discrete variables were presented as frequency and percentages. Variations were also compared with age, gender, ethnicity using independent T-test. Variations in morphology were also compared with hearing thresholds using Pearson correlation test. The p-value $\leq$ 0.05 considered to be statistically significant.

CHAPTER 4

RESULTS

A total of 50 participants were recruited for this study with 25 male and female each on the basis of five group's age. The parameters included were width of malleus head, length of manubrium, total length of malleus, width of incus and total length of incus. The mean value of malleus head width was 3.036 mm, length of manubrium was 4.472 mm and total length of malleus was 7.760 mm while mean value of Incus width was 4.440 mm and total length of incus was 6.604 mm observed in males. The mean value of width of the Malleus head was 3.000 mm, length of manubrium was 4.308 mm and total length of malleus was 7.412 mm while mean value of Incus width was 4.448 mm and total length of incus was 6.556 mm observed in females. These results showed that the morphological measurements in males were different and greater than in females (Table 4.1).

The shape of the malleus manubrium is mainly straight and curved in different individuals so according to the results the shape of manubrium (handle of malleus) was straight in 10 males and 8 in females. Curved manubrium was observed in 15 males and 17 female participants. As compared to females, males had more straight whereas females had more (17:15) curved handle of malleus (Table 4.2).

Hearing thresholds were observed in each individual according to gender 10-15dB and 20-30dB was observed in 7 males and 3 females. The threshold level of 10-20 dB and 30-40 dB was observed in 4 males and 6 females. The threshold level of 25-35 dB was observed in 3 males and 7 females (Table 4.3).

Comparison of ethnic groups with regards to width of malleus head showed that most significant novel findings in Sindhi which was present in 5 participants whose width was 3.0 mm, in Punjabi 3 participants had a width of 3.3 mm, in Balochi 2 participants had a width of 2.9 mm and 2 had 3.3 mm width, in Pashtun 3 participants had a width of 3.2 mm and the width of malleus head was 3 mm in Hindko (Figure 4.1).

Comparison of ethnic groups with length of manubrium (Malleus) showed that most significant novel findings in Sindhi and Pashtun were that 2 participants had a length of 4.4 mm and 2 participants had 4.6 mm length, in Punjabi 3 participants had 4.1 mm length, in Balochi 2 participants had 4.2 mm and in Hindko 3 participants had 4.4 mm length of malleus manubrium (Figure 4.2).

Comparison of ethnic groups showed a total length of malleus as the most significant novel findings in Sindhi 3 participants had 7.8 mm total length, in Punjabi 4 participants the length was 7.4 mm, in Pashtun 2 participants the length was 7.5 mm and 2 had 7.6 mm total length of malleus (Figure 4.3).

Comparison of ethnic groups with width of incus showed that most significant novel findings in Sindhi was in 5 participants that had 4.6 mm width, in Punjabi 2 participants had 4.1 mm width, 2 participants had 4.5 mm width and 2 participants had 4.8 mm width while in Pashtun 2 participants had a width of 4.6 mm and in Hindko 4 participants had 4.2 mm width of incus (Figure 4.4).

Comparison of ethnic groups with total length of incus showed that most significant novel findings in Sindhi was in 3 participants had a length of 6.4 mm and 3 had 6.5 mm length, in Punjabi 3 participants had a length of 6.3 mm, in Balochi 4 participants had a length of 6.4 mm, in Pashtun 3 participants had a length of 6.4 mm and in Hindko 3 participants had 6.7 mm total length of incus (Table 4.4).

Comparison of ethnic groups with shape of manubrium (straight/curved) showed that most significant novel findings in Sindhi and Punjabi was that 3 participants that had straight and 7 had curved shaped manubrium, in Balochi 1 participants had straight while 9 had curved shaped manubrium, in Pashtun 4 participants had straight and 6 had curved shaped manubrium while in Hindko 7 participants had straight and 3 had curved shaped manubrium of malleus (Table 4.5).

Comparison of ethnic groups with hearing thresholds showed that most significant novel findings in Sindhi and Punjabi was 10-15 dB, in Balochi was 20-30 dB, in Pashtun was 30-40 dB while in Hindko was 25-35 dB (Table 4.6). While shape of manubrium was found to be straight in 7 participants out of 18 in Hindko and curved in 9 participants out of 32 in Balochi ethnic groups (Table 4.5).

Age ranges were determined and results were analyzed in five groups divided as Group A: 10-20, Group B: 21-30, Group C: 31-40, Group D: 41-50, Group E: 51 years onward.

The results of the comparison of shape of manubrium (straight/curved) with all age groups showed that in (group A) total of 5 participants, in (group B) 2 participants, in (group C) 2 participants, in (group D) 6 participants and in (group E) 3 participants had straight manubrium while in (group A) total of 5 participants, in (group B) 8 participants, in (group C) 8 participants, in (group D) 4 participants and in (group E) 7 participants had curved manubrium (Table 4.7).

Between the groups the value of total length of incus had been observed to be statistically significant that is (0.0001) by applying Anova test (Table 4.8).

The results of the study showed that mean width of malleus head in group A was 2.91 mm, in B was 2.97 mm, in C was 3.09 mm, in D was 3.0 mm and in E was found to be 3.12 mm. The mean length of manubrium in group A was 4.32 mm, in B was 4.47 mm, in C was 4.43 mm, in D was 4.29 mm and in E was 4.44 mm. The mean total length of malleus was 7.47 mm in group A, 7.58 mm in group B, 7.67 mm in group C, 7.60 mm in group D and 7.61 mm in group E (Table 4.10). The mean width of incus in group A was 4.54 mm, in group B was 4.19 mm, in group C was 4.49 mm and in group D and E was 4.50 mm. The mean of total length of incus was 6.44 mm in group A, 6.37 mm in group B, 6.60 mm in group C, 6.71 mm in group D and 6.78 mm in group E was recorded (Table 4.11).

The mean width of malleus head in right ear was 3.035 mm while in left ear was 3.000 mm. The mean length of manubrium in right ear was 4.281 mm while in left ear was 4.508 mm. The mean total length of malleus was 7.477 mm in right ear while in left ear was 7.704 mm. The mean width of incus in right was 4.392 mm while in left ear was 4.500 mm and the total length of incus in right ear was 6.604 mm while in left was 6.554 mm (Table 4.9).

Out of 50 participants 12 had mean value of 3.02 mm for width of malleus head (Figure 4.5), 11 participants had mean value of 4.39 mm for malleus manubrium (Figure 4.6), 11 participants had mean value of 7.59 mm for total length of malleus (Figure 4.7), 9 participants had mean value of 4.44 mm for total width of incus (Figure 4.8 mm) and 12 participants had mean value of 6.58 mm for total length of incus (Figure 4.9).

Table 4.1 Morphological readings observed in male and female gender

Parameters	Gender (M/F)	N	Mean (mm)	Std. Deviation (±)
Malleus (width of head)	Male	25	3.036	0.3390
	Female	25	3.000	0.2814
length of manubrium	Male	25	4.472	0.4766
	Female	25	4.308	0.4499
Total length of malleus	Male	25	7.760	0.5979
	Female	25	7.412	0.5085
Total width of incus	Male	25	4.440	0.5196
	Female	25	4.448	0.4779
Total length of incus	Male	25	6.604	0.2406
	Female	25	6.556	0.1660

Table 4.2 Shape of manubrium (straight or curved) compared in male and female

SHAPE OF MANUBRIUM (Gender M/F)			Gender (M/F)		Total
			Male	Female	
Shape of manubrium	Straight	Count	10	8	18
		% within Gender (M/F)	40.0%	32.0%	36.0%
	Curved	Count	15	17	32
		% within Gender (M/F)	60.0%	68.0%	64.0%
Total		Count	25	25	50
		% within Gender (M/F)	100.0%	100.0%	100.0%

Table 4.3 Hearing thresholds compared with gender

HEARING THRESHOLDS (Gender M/F)			**Gender (M/F)**		**Total**
			Male	**Female**	
Hearing Thresholds(dB)	10-15	Count	7	3	10
		% within Gender (M/F)	28.0%	12.0%	20.0%
	20-30	Count	7	3	10
		% within Gender (M/F)	28.0%	12.0%	20.0%
	10-20	Count	4	6	10
		% within Gender (M/F)	16.0%	24.0%	20.0%
	25-35	Count	3	7	10
		% within Gender (M/F)	12.0%	28.0%	20.0%
	30-40	Count	4	6	10
		% within Gender (M/F)	16.0%	24.0%	20.0%
Total		Count	25	25	50
		% within Gender (M/F)	100.0%	100.0%	100.0%

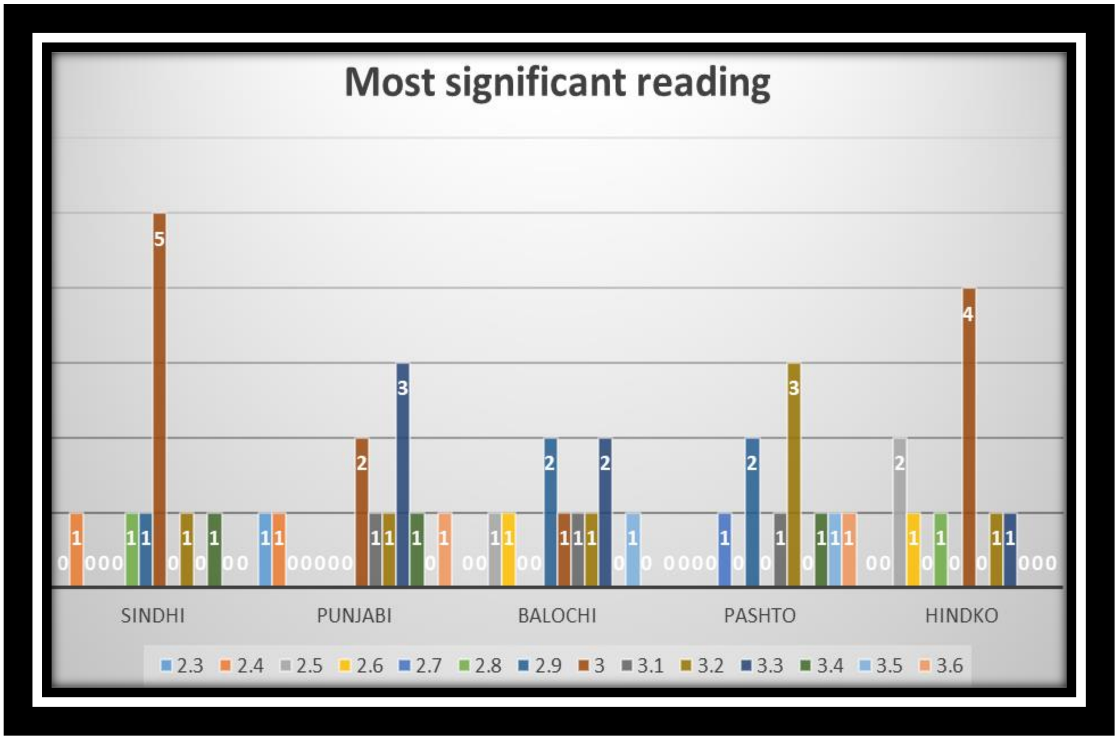


Figure 4.1 Comparison of ethnicity with width of malleus head (n= 50)

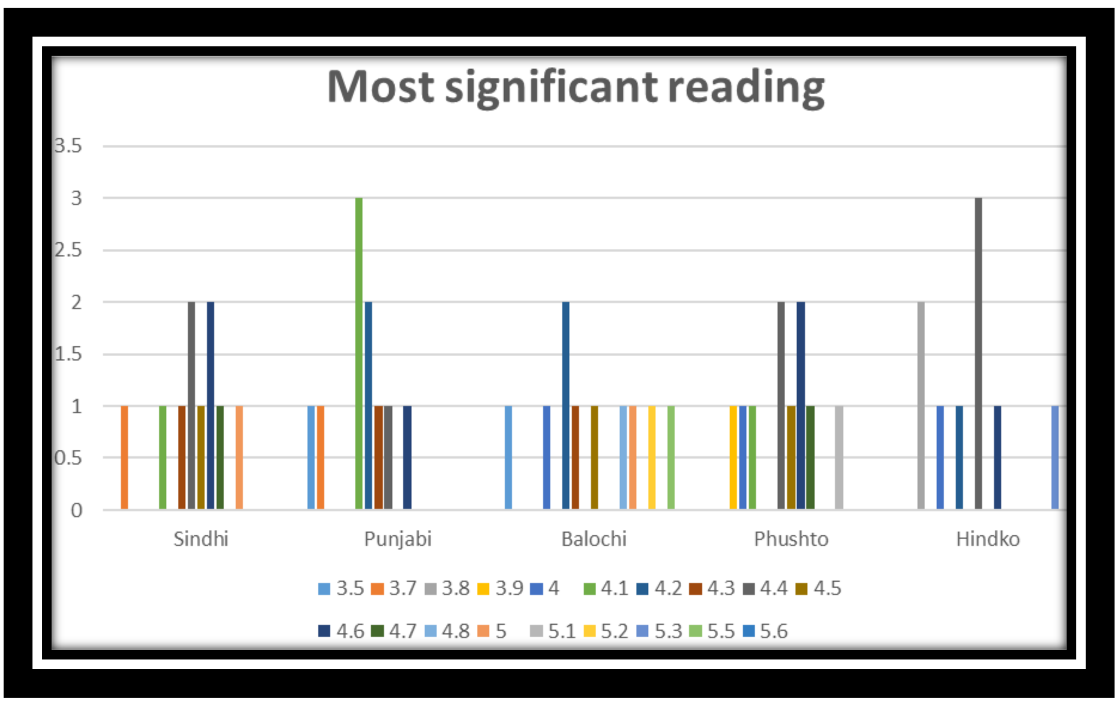


Figure 4.2 Ethnicity versus Length of manubrium (Malleus)

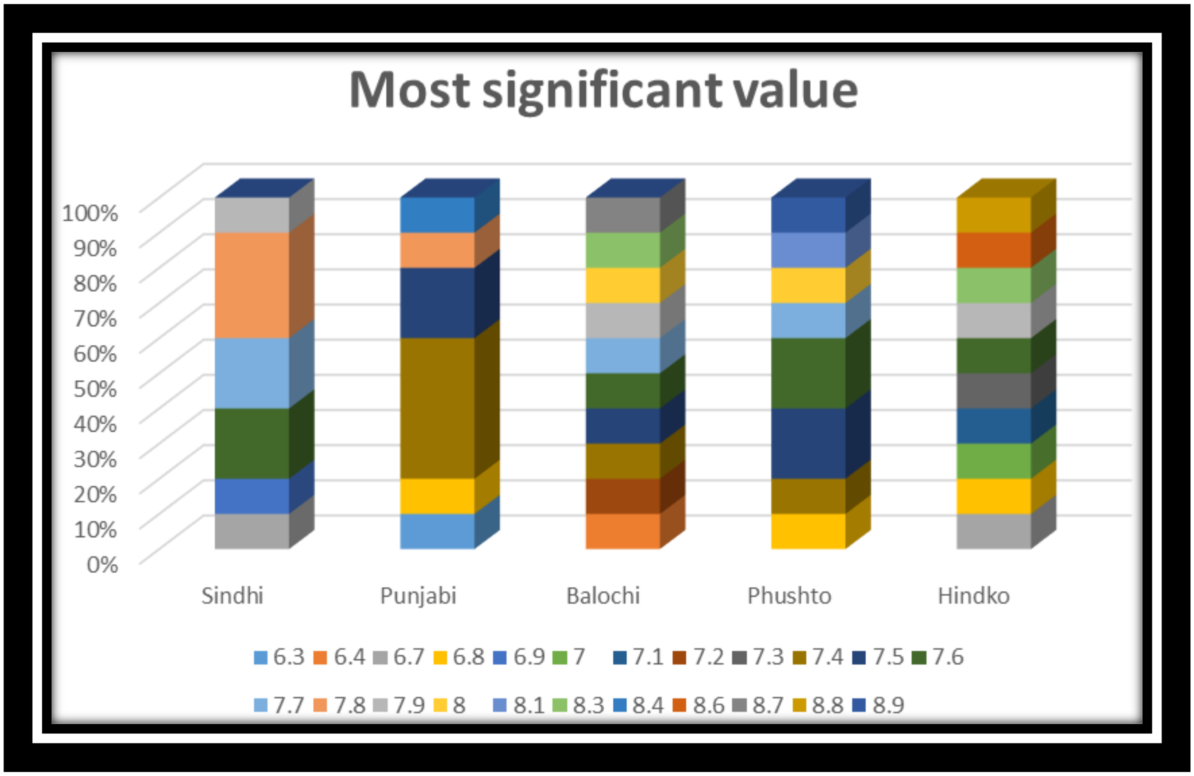


Figure 4.3 Ethnicity versus Total length of Malleus

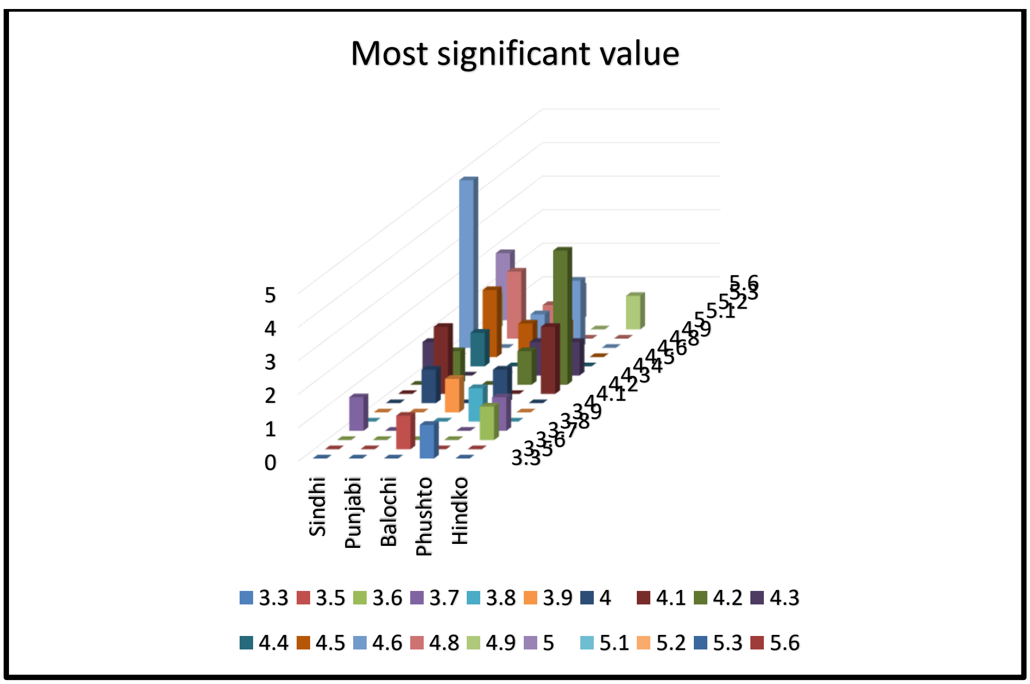


Figure 4.4 Ethnicity versus Width of Incus

Table 4.4 Ethnicity versus Total length of Incus

Ethnicity * Total length of incus Crosstabulation										
		Total length of incus								Total
ETHNICITY		6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.1	
Sindhi	Count	1	3	3	1	0	1	1	0	10
	% within Total length of incus	20.0%	25.0%	33.3%	16.7%	0.0%	16.7%	33.3%	0.0%	20.0%
Punjabi	Count	3	1	2	0	2	1	1	0	10
	% within Total length of incus	60.0%	8.3%	22.2%	0.0%	28.6%	16.7%	33.3%	0.0%	20.0%
Balochi	Count	1	4	1	1	1	2	0	0	10
	% within Total length of incus	20.0%	33.3%	11.1%	16.7%	14.3%	33.3%	0.0%	0.0%	20.0%
Pushto	Count	0	3	1	2	1	1	0	2	10
	% within Total length of incus	0.0%	25.0%	11.1%	33.3%	14.3%	16.7%	0.0%	100.0%	20.0%
Hindko	Count	0	1	2	2	3	1	1	0	10
	% within Total length of incus	0.0%	8.3%	22.2%	33.3%	42.9%	16.7%	33.3%	0.0%	20.0%
Total	Count	5	12	9	6	7	6	3	2	50
	% within Total length of incus	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 4.5 Ethnicity versus shape of manubrium (straight or curved)

Ethnicity * Shape of manubrium Crosstabulation				
ETHNICITY		Shape of manubrium		Total
		Straight	Curved	
Sindhi	Count	3	7	10
	% within Shape of manubrium	16.7%	21.9%	20.0%
Punjabi	Count	3	7	10
	% within Shape of manubrium	16.7%	21.9%	20.0%
Balochi	Count	1	9	10
	% within Shape of manubrium	5.6%	28.1%	20.0%
Pushto	Count	4	6	10
	% within Shape of manubrium	22.2%	18.8%	20.0%
Hindko	Count	7	3	10
	% within Shape of manubrium	38.9%	9.4%	20.0%
Total	Count	18	32	50
	% within Shape of manubrium	100.0%	100.0%	100.0%

Table 4.6 Comparison of ethnicity with different hearing threshold level

Ethnicity * Hearing threshold(dB) Crosstabulation							
ETHNICITY		Hearing threshold(dB)					Total
		10-15	20-30	10-20	25-35	30-40	
Sindhi	Count	4	2	2	2	0	10
	% within Hearing threshold(dB)	40.0%	20.0%	20.0%	20.0%	0.0%	20.0%
Punjabi	Count	4	1	2	0	3	10
	% within Hearing threshold(dB)	40.0%	10.0%	20.0%	0.0%	30.0%	20.0%
Balochi	Count	1	4	3	2	0	10
	% within Hearing threshold(dB)	10.0%	40.0%	30.0%	20.0%	0.0%	20.0%
Pushto	Count	1	2	1	2	4	10
	% within Hearing threshold(dB)	10.0%	20.0%	10.0%	20.0%	40.0%	20.0%
Hindko	Count	0	1	2	4	3	10
	% within Hearing threshold(dB)	0.0%	10.0%	20.0%	40.0%	30.0%	20.0%
Total	Count	10	10	10	10	10	50
	% within Hearing threshold(dB)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 4.7 Comparison of Age with Shape of manubrium

Age with shape of manubrium (straight/curved)								
			Age (yr)					Total
			10-20	21-30	31-40	41-50	51 onwards	
Shape of manubrium	Straight	Count	5	2	2	6	3	18
		% within Age (yr)	50.0%	20.0%	20.0%	60.0%	30.0%	36.0%
	Curved	Count	5	8	8	4	7	32
		% within Age (yr)	50.0%	80.0%	80.0%	40.0%	70.0%	64.0%
Total		Count	10	10	10	10	10	50
		% within Age (yr)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Table 4.8 Total length of Incus has been observed significant between age groups

ANOVA Test						
		Sum of Squares	df	Mean Square	F	Sig.
malleus (width of head)	Between Groups	0.299	4	0.075	0.768	0.551
	Within Groups	4.375	45	0.097		
	Total	4.674	49			
length of manubrium	Between Groups	0.254	4	0.064	0.275	0.893
	Within Groups	10.391	45	0.231		
	Total	10.645	49			
Total length of malleus	Between Groups	0.213	4	0.053	0.149	0.962
	Within Groups	16.087	45	0.357		
	Total	16.300	49			
Total width of incus	Between Groups	0.821	4	0.205	0.829	0.514
	Within Groups	11.142	45	0.248		
	Total	11.963	49			
Total length of incus	Between Groups	1.210	4	0.302	15.647	0.0001
	Within Groups	0.870	45	0.019		
	Total	2.080	49			

Table 4.9 Mean value of the parameters in relation to the side of the ear (right/left)

Means with side of the ear					
PARAMETERS	Side of the ear	N	Mean (mm)	Std. Deviation	Std. Error Mean
malleus (width of head)	Right	26	3.035	0.2966	0.0582
	Left	24	3.000	0.3270	0.0668
length of manubrium	Right	26	4.281	0.3960	0.0777
	Left	24	4.508	0.5141	0.1049
Total length of malleus	Right	26	7.477	0.5574	0.1093
	Left	24	7.704	0.5857	0.1196
Total width of incus	Right	26	4.392	0.5670	0.1112
	Left	24	4.500	0.4054	0.0828
Total length of incus	Right	26	6.604	0.2144	0.0421
	Left	24	6.554	0.1978	0.0404

Table 4.10 Means of malleus head width, length of manubrium and total length of malleus are appreciated in this table between age groups

		N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Malleus (width of head)	10-20	10	2.910	0.3446	2.663	3.157	2.3	3.4
	21-30	10	2.970	0.3974	2.686	3.254	2.4	3.6
	31-40	10	3.090	0.2767	2.892	3.288	2.5	3.4
	41-50	10	3.000	0.1826	2.869	3.131	2.6	3.2
	51 onwards	10	3.120	0.3155	2.894	3.346	2.5	3.5
	Total	50	3.018	0.3088	2.930	3.106	2.3	3.6
Length of manubrium	10-20	10	4.320	0.4158	4.023	4.617	3.5	5.0
	21-30	10	4.470	0.6183	4.028	4.912	3.7	5.6
	31-40	10	4.430	0.4877	4.081	4.779	3.7	5.2
	41-50	10	4.290	0.5280	3.912	4.668	3.5	5.3
	51 onwards	10	4.440	0.2875	4.234	4.646	4.1	5.1
	Total	50	4.390	0.4661	4.258	4.522	3.5	5.6
Total length of malleus	10-20	10	7.470	0.5165	7.101	7.839	6.3	8.0
	21-30	10	7.580	0.5007	7.222	7.938	6.7	8.6
	31-40	10	7.670	0.5618	7.268	8.072	6.8	8.7
	41-50	10	7.600	0.7118	7.091	8.109	6.4	8.8
	51 onwards	10	7.610	0.6691	7.131	8.089	6.7	8.9
	Total	50	7.586	0.5768	7.422	7.750	6.3	8.9

Table 4.11 Means of Incus width and total length of incus are appreciated in this table between age groups

		2N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
					Upper limit	Lower limit		
Total width of incus	10-20	10	4.540	0.3627	4.281	4.799	4.0	5.2
	21-30	10	4.190	0.4280	3.884	4.496	3.5	4.6
	31-40	10	4.490	0.4932	4.137	4.843	4.0	5.6
	41-50	10	4.500	0.4761	4.159	4.841	3.7	5.1
	51 onwards	10	4.500	0.6733	4.018	4.982	3.3	5.3
	Total	50	4.444	0.4941	4.304	4.584	3.3	5.6
Total length of incus	10-20	10	6.440	0.1430	6.338	6.542	6.3	6.8
	21-30	10	6.370	0.0483	6.335	6.405	6.3	6.4
	31-40	10	6.600	0.1155	6.517	6.683	6.5	6.8
	41-50	10	6.710	0.1197	6.624	6.796	6.5	6.9
	51 onwards	10	6.780	0.2150	6.626	6.934	6.5	7.1
	Total	50	6.580	.2060	6.521	6.639	6.3	7.1

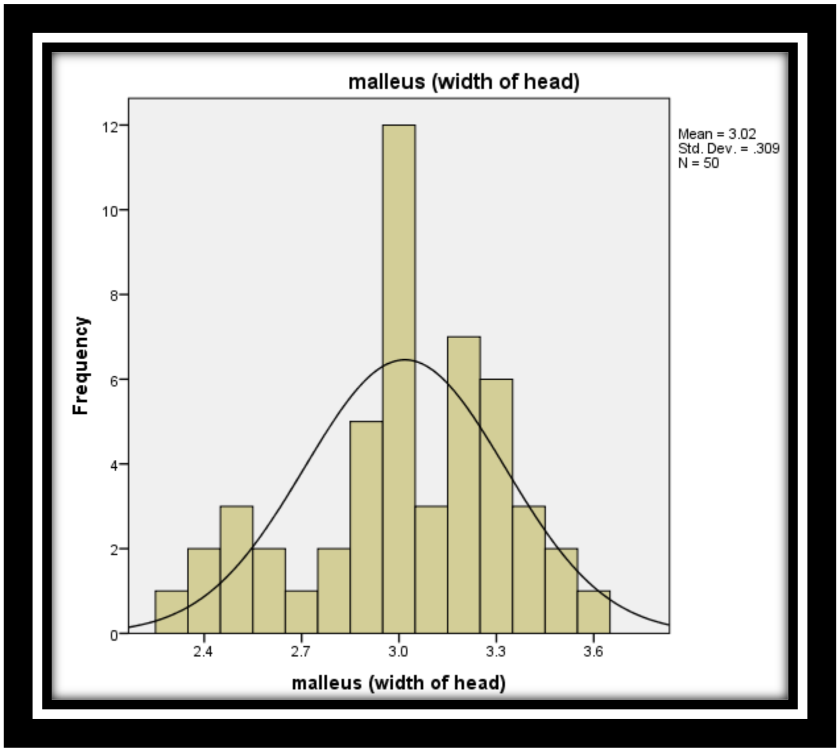


Figure 4.5 Frequency chart showing mean width of malleus head

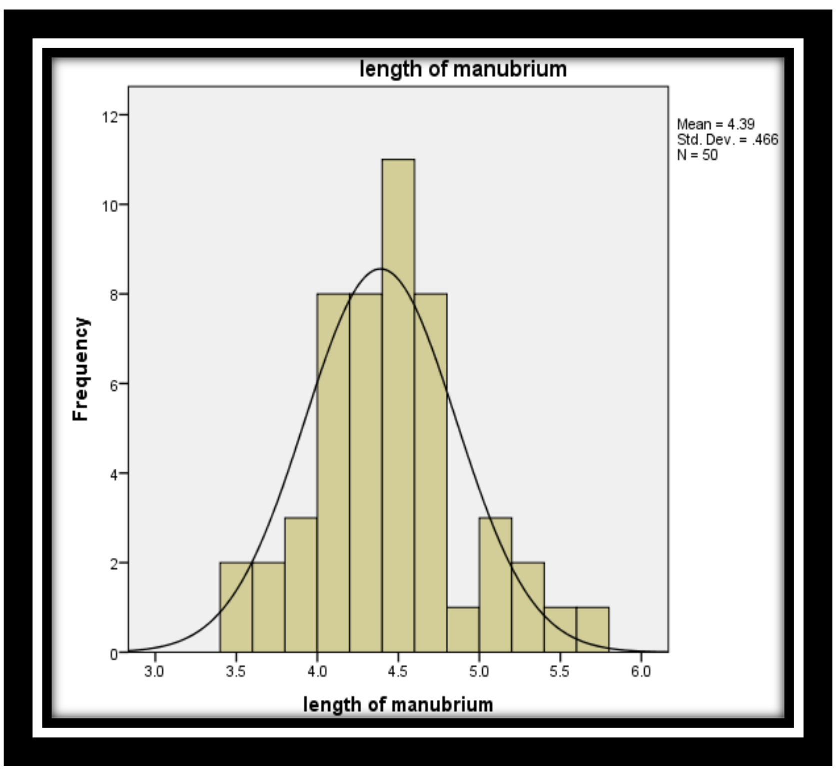


Figure 4.6 Frequency chart showing mean length of manubrium

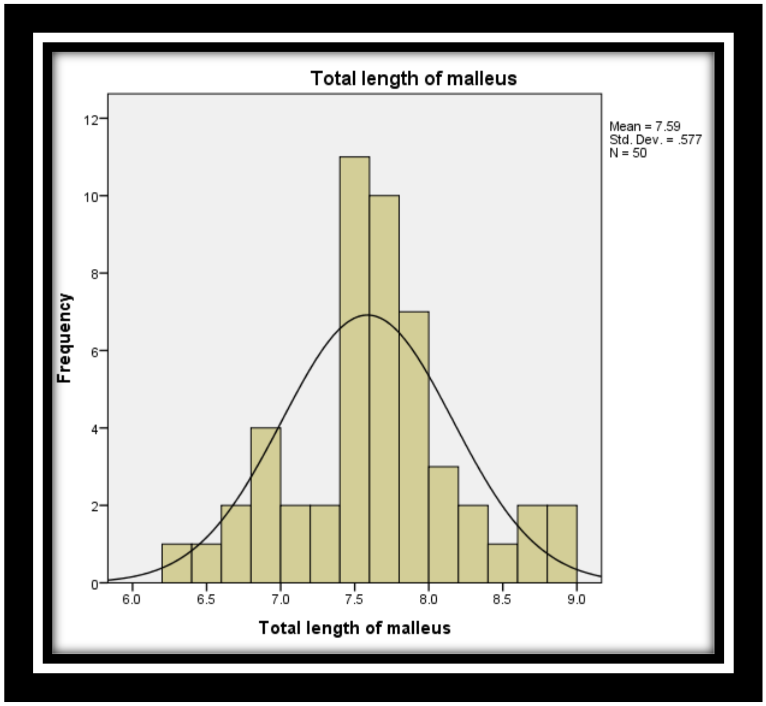


Figure 4.7 Frequency chart showing mean total length of malleus

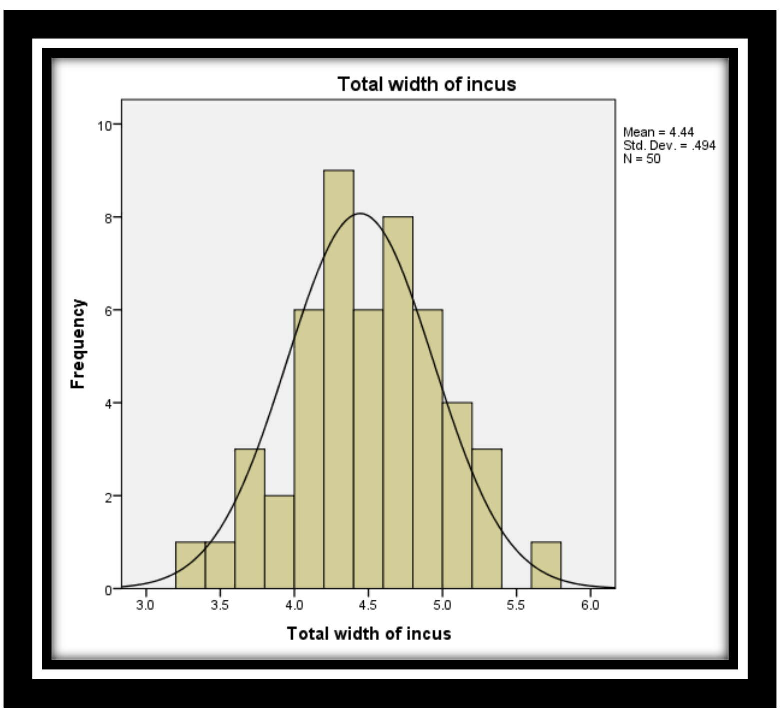


Figure 4.8 Frequency chart showing mean total width of incus

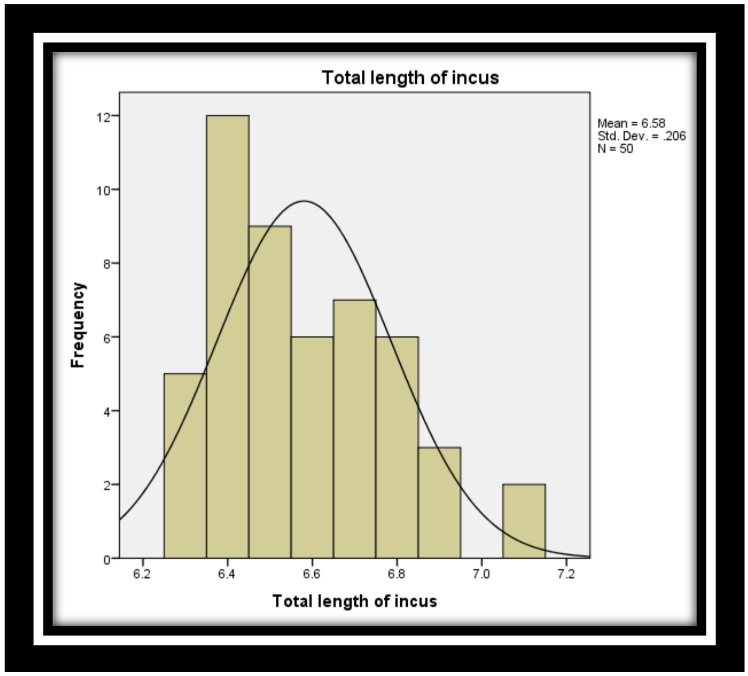


Figure 4.9 Frequency chart showing mean total length of incus

CHAPTER 5

DISCUSSION

Literature search shows that morphometric data obtained can be useful for the reconstructive procedures. Preoperative radiological assessment is advised for these small bones. Some of areas in these bones are at great risk for injuries with consequential intraoperative complications. Hence the knowledge of anatomical variations of ear ossicles is crucial for ENT specialists as well as for radiologists for preoperative evaluation of these varieties to avoid iatrogenic complications after transplants and identify the difference between normal morphology and eroded ossicles due to trauma (Delrue et al., 2016).

Reliability of high-resolution CT scan in diagnosis of ossicular tympanosclerosis and all the diseases leading to erosion of ossicles is considered as the gold standard radiological investigation for assessment of middle ear cavity. There are many anatomic variants in petrous temporal region which can be easily detected by CT scan (Larem et al., 2021).

Variation in these bones have been noted previously mostly through cadaveric studies which is expensive and time consuming related to the changes. A CT evaluation has been moderately done for diseases related to middle ear cavity and it has been noted that this standard investigation is the fastest way to detect these changes by taking measurements in console CT rooms with the help advanced software. The cause of these variations may be due to amount of bony tissue located in that particular area of bone, extension and direction of changes in bone growth, remodeling process as different parts of ossicles shows different rate of growth in each individual.

Kumar et al., in 2018 reported in a morphometric study of human middle ear ossicles in cadaveric temporal bones of Indian population that the mean total length of the malleus was 8.23 mm, mean width of the head 2.56 mm, and mean length of manubrium was 4.17 mm. The mean total length of incus was 7.04 mm and mean total width of incus was 5.31 mm noted while in the present study it was reported that the mean total length of the malleus was 7.59 mm, mean width of the head was 3.02 mm, and mean length of manubrium was 4.39 mm. The mean total length of incus was 6.57 mm, and mean total width of incus was 4.44 mm. The variations seen in malleus were mainly due to presence and absence of neck in malleus while the depression in the articulating surface of incus and bony outgrowth on lenticular process may be the cause of variations in incus.

Malleus handle also known as manubrium showed variation in morphology in individuals in terms of shape, it was seen that malleus manubrium had straight and curved processes (Sodhi et al., 2017). In this present study out of 50 participants 18 had straight while 32 had curved manubrium.

A meta-analysis of 50 year literature showed varying values of malleus head width, manubrium length, total length of malleus, width of incus and total length of incus (Table 5.1 and 5.2).

The middle ear ossicles form a semi rigid chain in the middle of the ear for conduction and amplification of sound waves from the tympanic membrane to the inner ear. Although, our knowledge of the ear ossicles dates back to the 15th century, and various studies have been carried out on their morphometry, morphology, anomalies, embryology, function and structure throughout the world, information about the morphology of middle ear ossicles is meagre in Indian subjects. Malleus presented variations in the free ends of manubrium, lateral process and anterior process (Saha et al., 2017).

In 2018, in order to determine the normal range of the values of various measurements of malleus and whether these measurements are useful for the sexual dimorphism or not. Out of total 60 malleus, 30 were retrieved from the male cadavers and 30 were retrieved from the female cadavers. Mean of total length of malleus, mean of length of manubrium of malleus and mean of length of head and neck of malleus were observed to be more in males as compared to female (Javia et al., 2018). Similarly in the present study mean of total length of malleus, length of manubrium of malleus and mean of width of malleus head were observed to be more in male as compared to female. It was concluded that due to difference in body mass index (BMI) between male and females, the values for male gender were greater compared to females as in males BMI was noted to be greater in them.

Parameters	Singh et al, 2012 right	Singh et al, 2012 left	Unur et al, 2002	Vinayachandra et al, 2014	Mogra et al, 2014	Harneja & Chaturvedi, 1973	Arensburg et al, 1981
Total length	7.947	7.9467	7.69	7.45	8.53	7.15	7.8
Length of manubrium	4.762	4.726	4.70	-	4.22	4.22	4.4

Table 5.1 Mean values of malleus

Parameters	Unur et al, 2002	Harneja & Chaturvedi	Arensburg et al, 1981
Total length	6.47	3.14	6.4
Total width	4.88	1.82	-

Table 5.2 Mean values of incus

The ossicular chain can be partially destroyed by inflammatory diseases such as chronic suppurative otitis media or cholesteatoma. Ossiculoplasty, or reconstruction of the middle-ear bones, has previously been achieved using a variety of methods, from auto graft or homograft ossicles to prosthetic implants. Early work used bone chips to reconnect the eroded incus long process to the stapes head (Misurya et al., 1980).

An ideal prosthesis for ossiculoplasty in cases where any bone is absent or defective would be one that replicated the acoustic properties of a properly functioning bone and integrated acoustically within the normal ossicular chain. The intact middle ear is the standard that any reconstruction must aim to achieve. An anatomically shaped bone prosthesis, appropriately attached, may transmit vibration in a manner similar to that of the original ossicle. Clinical success has been reported by one of the authors in adopting a physiological approach to ossicular reconstruction in a small number of patients with defects of ossicles, utilizing a small cortical bone graft (Cox et al., 2017).

The distal part of manubrium mallei showed variations in being curved anteriorly or being straight. Some mallei had no neck between head and manubrium. One malleus showed large rounded head and one malleus had a bony process projected from its head. The average of morphometric parameters showed that the malleus was 8.53mm in total length, the manubrium mallei was 5.20mm, and the total length of head and neck was 4.72mm. No significant difference was found when they compared these 3 parameters of right and left side (Mogra et al., 2014).

In this present study novel findings were observed in morphometric parameters when sides of the ear were compared which showed mean width of malleus head and mean total length of incus were more in right side as compared to left while the mean length of manubrium, mean total length of malleus, mean length of manubrium and mean width of incus was found to be more in left side of the ear then right side.

(Nadeem, 2012) explained by taking 22 fetal cadavers of either sex, ranging in gestational ages between 24-40 week and 15 adult formalin-fixed cadavers in age groups of 20-30 years. For the study, the fetuses were kept in 2 Groups of 24-28 weeks and 36-40 weeks. From the data obtained, the mean, standard deviation and percentage gain from one group to another in different dimensions of various parts of each ossicle were calculated. The results showed that the age period between 40 weeks (full term fetuses) and adults, revealed spurt in the growth of diameter of head of malleus (3%), width of short process of incus (2.7%), width of footplate (5.2%) and height of stapes (13%). These changes were insignificant in terms of acoustic transmission but it did show morphological variations.

The present study was conducted to compare the morphological changes in ear ossicles with different age groups, in order to identify the pattern of variations in terms of morphology that is shape and size. The mean of all 5 parameters taken into account (width of malleus head, length of manubrium, and total length of malleus, total length of incus and width of incus) showed variations in terms of measurements taken (mean) and shape or form of ossicles.

The pure-tone audiogram provides information as to hearing sensitivity across a selected frequency range. Normal or near-normal pure-tone thresholds sometimes are observed despite cochlear damage. There are a surprising number of patients with acoustic neuromas who have essentially normal pure-tone thresholds. In cases of central deafness, depressed pure-tone thresholds may not accurately reflect the status of the peripheral auditory system. Listening difficulties are seen in the presence of normal pure-tone thresholds. Suprathreshold procedures and a variety of other tests can provide information regarding other and often more central functions of the auditory system (Musiek et al., 2017).

The present study compared the hearing threshold levels with age, gender, side of the ear and ethnicity in relation to their mean values of 5 parameters (width of malleus head, length of manubrium, and total length of malleus, total length of incus and width of incus) and variations were found to be significant.

CHAPTER 6

CONCLUSION

6.1 Conclusion of the study

On the basis of this study, it can be concluded that there are morphological variations in ear ossicles in terms of size and shape in correlation with age, gender, ethnicity and side of the ear. These variations although have no effect on differences in hearing thresholds, it itself varies among individuals of different age, gender and ethnicity.

The most frequent anatomical variation was seen in malleus manubrium with straight and curved processes. The total length of incus was found to be significant among all parameters. In accordance to gender means of all 5 parameters (width of malleus head, length of manubrium, and total length of malleus, total length of incus and width of incus) were found to be greater in male than female.

The evaluation of these bones were mostly done in cadaveric study, this study concluded that HRCT scan of petrous temporal bone is an innovative and latest technique which can be used to measure and note these anatomical changes in such a minute structure that is ear ossicles.

6.2 Recommendations

In this era, it is very important to have precise knowledge about variations in complex anatomical structures which is essential for surgeons. The cost of any investigation and research needs to be evaluated for growth purposes. HRCT- PTB scan provides an accurate, cost effective, fastest and easiest method for identification of the normal morphology, anatomical variants and the extent of disease in the middle- ear cavity and areas around it.

In addition to variations in malleus and incus which were considered in this study, according to literature review there were also morphological variation seen in stapes which needs to be evaluated in future. Further investigations like ultrasound can be compared with CT scan technique in order to draw advantages and disadvantages of both.

The information about these bones is meagre to record and mainly cadaveric, so it needs to be researched further to enhance and increase the literature record which will help in establishing of an accurate prosthesis in future surgeries.

6.3 Strengths of the study

This study highlighted the importance of CT scan evaluation of various anatomical variations of ear ossicles. It compared all variations among age, gender, and ethnicity. This study give a clue that variations were more noticed in male gender. It also helped us to determine that in case of prosthetic procedures of this structure, every individual has different size and shape of bone in terms of anatomy. A pre-radiological investigation with measurements should be taken while constructing and placing the prosthesis and auto grafts for these structures in surgeries to avoid any future mishaps.

Knowing the morphometric anatomy of ossicles has become very important in otologic surgeries. In any ear pathology the disease can erode the ossicles causing hearing loss. Morphometric data is useful for the reconstructive procedures. Preoperative radiological assessment is advised for these small bones. It also emphasizes on the future directions where in reconstructive procedures can be improved with the artistic renderings of the blueprints provided, for new prosthetic designs which can be manufactured by using Teflon materials.

It will help in planning Malleostapedopexy, Incudostapedopexy, and stapedopexy procedures. Various anomalies of these ossicles can lead to the hearing problems. Various congenital malformations of these ossicles have also been reported to cause hearing problems. To perform the microsurgical maneuvers and manipulations, so as to correct these abnormalities, surgeons need to be fully conversant about the details of these tiny bones. So, the study was designed to report about the various variations of the ossicles which would help them while performing the reconstructive surgeries.

This study also stressed on the clarity and the answer to the question that whether there are variations present or not.

6.4 Limitations of the study

Larger population size and multicenter options must be taken in order to authenticate study further. All anatomical variations mentioned in the literature are not included in this study. The present study did not look for association of variations in ear ossicles with hearing thresholds due to restricted time period for study completion. The size of ear ossicles is very minute although multiple readings were taken with the help of software and mean was drawn to decrease human error but there may be chances of its presence.

Selection bias criteria was used while including the participants in this study it may cause the results to not been considered as generalized to a population.

CHAPTER 7

REFERENCES

- Abu-Naser, S. S., & Abu Hasanein, H. A. (2016). Ear Diseases Diagnosis Expert System Using SL5 Object.
- Adegbiji, W. A., Olajide, G. T., Olajuyin, O. A., Olatoke, F., Alabi, S. A., & Nwawolo, C. C. (2018). Traumatic tympanic membrane perforation in Tertiary Health Institution. *The Journal of Middle East and North Africa Sciences*, *10*(5637), 1-7.
- Aernouts, J., Aerts, J. R., & Dirckx, J. J. (2012). Mechanical properties of human tympanic membrane in the quasi-static regime from in situ point indentation measurements. *Hearing research*, *290*(1-2), 45-54.
- Alberti, P. W. (2001). The anatomy and physiology of the ear and hearing. *Occupational exposure to noise: Evaluation, prevention, and control*, 53-62.
- Ansen S, Peters N, Hinkelbein J, Klußmann JP, Beutner D, Meyer MF. Subjective and Objective Effectiveness of Eustachian Tube Balloon Dilatation for Patients With Eustachian Tube Dysfunction-Evaluation in a Pressure Chamber. Otol Neurotol. 2020 Jul; 41(6):795-801.
- Anthwal, N., & Thompson, H. (2016). The development of the mammalian outer and middle ear. *Journal of anatomy*, *228*(2), 217-232.
- Anwar, K., Khan, S., & Khan, S. (2019). Frequency of Type of Hearing Loss in Patients Presenting to ENT Outpatient Clinics. *Journal of Saidu Medical College*, *9*(1).
- Atmaja, B. T. (2021). The Physiology, Mechanism, and Nonlinearities of Hearing.
- Bartling, M. L., Rohani, S. A., Ladak, H. M., & Agrawal, S. K. (2021). Micro-CT of the human ossicular chain: Statistical shape modeling and implications for otologic surgery. *Journal of Anatomy*.
- Bhatnagar, D. P., Singhal, P., & Thapar, S. P. (2001). Anatomy of malleus–A human ear ossicle. *The Anthropologist*, *3*(2), 139-141.
- Brownell W. E. (1997). HOW THE EAR WORKS - NATURE'S SOLUTIONS FOR LISTENING. *The Volta review*, *99*(5), 9–28.
- Butt, M. F., Albusoda, A., Farmer, A. D., & Aziz, Q. (2020). The anatomical basis for transcutaneous auricular vagus nerve stimulation. *Journal of anatomy*, *236*(4), 588-611.
- Buytaert, J. A., Van der Jeught, S., Ars, B., Podoleanu, A. G. H., & Dirckx, J. J. J. (2013). Preliminary human tympanic membrane thickness data from optical coherence tomography. Opt Meas Tech Struct Syst Shaker Publishing 75–84.
- Christov, F., Nelson, E. G., Xu, L. J., Lopez, I. A., Ishiyama, A., & Gluth, M. B. (2020). Histology of the Cochlear Outer Sulcus Cells in Normal Human Ears, Presbycusis, and Menière's Disease. *Otology & Neurotology*, *41*(4), e507-e515.

- Costeur, L., Mennecart, B., Müller, B., & Schulz, G. (2017). Prenatal growth stages show the development of the ruminant bony labyrinth and petrosal bone. *Journal of Anatomy*, *230*(2), 347-353.
- Cox, M. D., Trinidade, A., Russell, J. S., & Dornhoffer, J. L. (2017). Long-term hearing results after ossiculoplasty. *Otology & Neurotology*, *38*(4), 510-515.
- Davis, H., & Silverman, S. R. (1970). Hearing and deafness. Holt, Rinehart & Winston of Canada Ltd.
- Dawood, M. R. (2017). Frequency dependence hearing loss evaluation in perforated tympanic membrane. *International archives of otorhinolaryngology*, *21*(4), 336-342.
- De Greef, D., Buytaert, J. A., Aerts, J. R., Van Hoorebeke, L., Dierick, M., & Dirckx, J. (2015). Details of human middle ear morphology based on micro‐CT imaging of phosphotungstic acid stained samples. *Journal of morphology*, *276*(9), 1025-1046.
- Delrue, S., Verhaert, N., van Dinther, J., Zarowski, A., Somers, T., Desloovere, C., & Offeciers, E. (2016). Surgical management and hearing outcome of traumatic ossicular injuries. *The journal of international advanced otology*, *12*(3), 231.
- Dhingra, P. L., & Dhingra, S. (2017). *Diseases of Ear, Nose and Throat-eBook*. Elsevier India.
- Ediale, J., Adobamen, P. R., & Ibekwe, T. S. (2018). Audiometric assessment of adolescents and adults with tympanic membrane perforation in Benin City. *Int J Otorhinolaryngol Head Neck Surg*, *4*(4), 901-906.
- Engström, H., Ades, H. W., & Hawkins, J. E. (1964). Cytoarchitecture of the organ of Corti. *Acta Oto-Laryngologica*, *57*(sup188), 92-99.
- Entsfellner, K., Kuru, I., Strauss, G., & Lueth, T. C. (2015, December). A new physical temporal bone and middle ear model with complete ossicular chain for simulating surgical procedures. In *2015 IEEE International Conference on Robotics and Biomimetics (ROBIO)* (pp. 1654-1659). IEEE.
- Gardner, M. M., Hickmott, J., & Ludvik, M. J. B. (2012). *Demonstrating student success: A practical guide to outcomes-based assessment of learning and development in student affairs*. Stylus Publishing, LLC.
- Gaur, S., Sinha, O. N., Bhushan, A., & Batni, G. (2017). Observations on tympanic membrane perforations (safe type) and hearing loss. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *69*(1), 29-34.
- Giuliano, K., & Pozzar, R. (2021). Achieving Noise Reduction With a Novel Lower Limb External Mechanical Compression System. *Ergonomics in Design*, 10648046211016690.
- Glynn, M., & Drake, W. M. (2017). Hutchison's Clinical Methods E-Book: An Integrated Approach to Clinical Practice. Elsevier Health Sciences
- Glynn, M., & Drake, W. M. (2017). *Hutchison's Clinical Methods E-Book: An Integrated Approach to Clinical Practice*. Elsevier Health Sciences.
- Green Jr, J. D., Shelton, C., & Brackmann, D. E. (1994). Iatrogenic facial nerve injury during otologic surgery. *The Laryngoscope*, *104*(8), 922-926.

- Gulati, M., Gupta, S., Prakash, A., Garg, A., & Dixit, R. (2019). HRCT imaging of acquired cholesteatoma: a pictorial review. *Insights into imaging*, *10*(1), 1-8.
- Gupta, A., Gupta, A., Jain, K., & Gupta, S. (2018). Noise pollution and impact on children health. *The Indian Journal of Pediatrics*, *85*(4), 300-306.
- Hentzer, E. (1970). Histologic studies of the normal mucosa in the middle ear, mastoid cavities and Eustachian tube. *Annals of Otology, Rhinology & Laryngology*, *79*(4), 825-833.
- Iskander, A., Naftalovich, R., & Pahlevan, N. M. (2020). The Carotid Sinus as a Viscometer. *Diagnostics*, *10*(11), 924.
- Iversen, M. M., & Rabbitt, R. D. (2017). Wave mechanics of the vestibular semicircular canals. *Biophysical journal*, *113*(5), 1133-1149.
- Javia, M., & Saravanan, P. (2020). Morphometric analysis of various measurements of malleus on the basis of sexual dimorphism. *IP Indian Journal of Anatomy and Surgery of Head, Neck and Brain*, *4*(4), 94-101.
- Kappel, S. L., Makeig, S., & Kidmose, P. (2019). Ear-EEG forward models: improved head-models for ear-EEG. *Frontiers in neuroscience*, *13*, 943.
- Kist, M., & Scheifele, P. M. (2019). Photomicrography of the middle ear ossicles. *The Journal of the Acoustical Society of America*, *145*(3), 1799-1799.
- Kumar, D. V., Chaitanya, D. K., Singh, V., & Reddy, D. S. (2018). A morphometric study of human middle ear ossicles in cadaveric temporal bones of Indian population and a comparative analysis. *Journal of the Anatomical Society of India*, *67*(1), 12-17.
- Kuru, I., Maier, H., Müller, M., Lenarz, T., & Lueth, T. C. (2016). A 3D-printed functioning anatomical human middle ear model. *Hearing Research*, *340*, 204-213.
- Landry, T. G., Rainsbury, J. W., Adamson, R. B., Bance, M. L., & Brown, J. A. (2015). Real-time imaging of in-vitro human middle ear using high frequency ultrasound. Hearing research, 326, 1-7.
- Larem, A., Abu Rajab Altamimi, Z., Aljariri, A. A., Haidar, H., Elsotouhy, A., Alsaadi, A., & Alqahtani, A. (2021). Reliability of high-resolution CT scan in diagnosis of ossicular tympanosclerosis. *Laryngoscope Investigative Otolaryngology*.
- Leblanc, A. (2013). *Atlas of hearing and balance organs: a practical guide for otolaryngologists*. Springer Science & Business Media.
- Li, J., Chen, K., Li, C., Yin, D., Zhang, T., & Dai, P. (2017). Anatomical measurement of the ossicles in patients with congenital aural atresia and stenosis. International journal of pediatric otorhinolaryngology, 101, 230-234.
- Liaw, J., Patel, V. A., & Carr, M. M. (2017). Congenital anomalies of the external ear. *Operative Techniques in Otolaryngology-Head and Neck Surgery*, *28*(2), 72-76.
- Lim, R., & Brichta, A. M. (2016). Anatomical and physiological development of the human inner ear. *Hearing research*, *338*, 9-21.
- Lindsey, B. D., Shelton, S. E., Martin, K. H., Ozgun, K. A., Rojas, J. D., Foster, F. S., & Dayton, P. A. (2017). High resolution ultrasound superharmonic perfusion

imaging: In vivo feasibility and quantification of dynamic contrast-enhanced acoustic angiography. Annals of biomedical engineering, 45(4), 939-948.

- Lucidi, D., De Corso, E., Paludetti, G., & Sergi, B. (2019). Quality of life and functional results in canal wall down vs canal wall up mastoidectomy. *Acta Otorhinolaryngologica Italica*, *39*(1), 53.
- Lukolo, L. N., Kimera, L. C., & Pilbee, G. (2021). Self-Ear Cleaning Practices and the Associated Risks: A Systematic Review. *Global Journal of Health Science*, *13*(5), 1-44.
- Lyu, H. Y., Chen, K. G., Yin, D. M., Hong, J., Yang, L., Zhang, T. Y., & Dai, P. D. (2016). The age-related orientational changes of human semicircular canals. Clinical and experimental otorhinolaryngology, 9(2), 109.
- Mackowetzky, K., Yoon, K. H., Mackowetzky, E. J., & Waskiewicz, A. J. (2021). Development and evolution of the vestibular apparatuses of the inner ear. *Journal of Anatomy*.
- Mammano, F. (2018). Inner ear connexin channels: roles in development and maintenance of cochlear function. *Cold Spring Harbor perspectives in medicine*, a033233.
- Mason, M. J. (2016). Structure and function of the mammalian middle ear. II: Inferring function from structure. *Journal of anatomy*, *228*(2), 300-312.
- Mescher, A. L. (2013). *Junqueira's basic histology: text and atlas* (Vol. 12). 13th Ed. New York: McGraw-Hill Medical.
- Mogra, K., Gupta, S., Chauhan, S., & Panwar, L. (2014). Morphological and morphometrical variations of malleus in human cadavers. *International J of Healthcare and Biomedical Research*, *2*, 186-192.
- Moore, K. L., & Dalley, A. F. (2018). *Clinically oriented anatomy*. Wolters kluwer india Pvt Ltd.
- Moore, K. L., Persaud, T. V. N., & Torchia, M. G. (2018). *The Developing Human-E-Book: Clinically Oriented Embryology*. Elsevier Health Sciences.
- Musiek, F. E., Shinn, J., Chermak, G. D., & Bamiou, D. E. (2017). Perspectives on the pure-tone audiogram. *Journal of the American Academy of Audiology*, *28*(07), 655-671.
- Musiek, F. E., Shinn, J., Chermak, G. D., & Bamiou, D. E. (2017). Perspectives on the pure-tone audiogram. *Journal of the American Academy of Audiology*, *28*(07), 655-671.
- Nadeem, G. (2012). Can fetal ossicles be used as prosthesis in adults? A morphometric study. *Anatomy*, *7*(1).
- Nadeem, G. (2012). Can fetal ossicles be used as prosthesis in adults? A morphometric study. *Anatomy*, *7*(1).
- Niekrash, C. E. (2021). Anatomy of the External Ear. In *Applied Head and Neck Anatomy for the Facial Cosmetic Surgeon* (pp. 85-89). Springer, Cham.

- Pagano, A. S., Trippiedi, A., Durrant, F., & Marquez, S. Do morphological differences in orientation of the Eustachian tube and dilator tubae muscle distinguish populations with high rates of middle ear disease?. *The FASEB Journal*, *35*.
- Pedler, K., & Chang, P. (2005). Ear examination: a practical guide. *Australian family physician*, *34*(10).
- Pitris, C., Saunders, K. T., Fujimoto, J. G., & Brezinski, M. E. (2001). High-resolution imaging of the middle ear with optical coherence tomography: a feasibility study. *Archives of Otolaryngology–Head & Neck Surgery*, *127*(6), 637-642.
- Priyadharshini, R. A., Arivazhagan, S., & Arun, M. (2021). A deep learning approach for person identification using ear biometrics. *Applied Intelligence*, *51*(4), 2161-2172.
- Purcell, P. L., Jones-Goodrich, R., Wisneski, M., Edwards, T. C., & Sie, K. C. (2016). Hearing devices for children with unilateral hearing loss: Patient-and parent-reported perspectives. *International journal of pediatric otorhinolaryngology*, *90*, 43-48.
- Quam, R. M., Coleman, M. N., & Martínez, I. (2014). Evolution of the auditory ossicles in extant hominids: metric variation in African apes and humans. *Journal of anatomy*, *225*(2), 167-196.
- Quesnel, S., Benchaa, T., Bernard, S., Martine, F., Viala, P., Van Den Abbeele, T., & Teissier, N. (2015). Congenital middle ear anomalies: anatomical and functional results of surgery. *Audiology and Neurotology*, *20*(4), 237-242.
- Rolvien, T., Schmidt, F. N., Milovanovic, P., Jähn, K., Riedel, C., Butscheidt, S., & Busse, B. (2018). Early bone tissue aging in human auditory ossicles is accompanied by excessive hypermineralization, osteocyte death and micropetrosis. Scientific reports, 8(1), 1-11.
- Saha, R., Srimani, P., Mazumdar, A., & Mazumdar, S. (2017). Morphological variations of middle ear ossicles and its clinical implications. *Journal of clinical and diagnostic research: JCDR*, *11*(1), AC01.
- Shapiro, N. L. (Ed.). (2011). *Handbook of pediatric otolaryngology: a practical guide for evaluation and management of pediatric ear, nose, and throat disorders*. World Scientific.
- Snell, R. S. (2018). *Snell's Clinical Anatomy*. Wolters kluwer india Pvt Ltd.
- Sodhi, S., Singh, Z., & Davessar, J. L. (2017). A study on morphological variations of middle ear ossicles. *Journal of Advanced Medical and Dental Sciences Research*, *5*(5), 1.
- Stoessel, A., David, R., Gunz, P., Schmidt, T., Spoor, F., & Hublin, J. J. (2016). Morphology and function of Neandertal and modern human ear ossicles. *Proceedings of the National Academy of Sciences*, *113*(41), 11489-11494.
- Sundar, P. S., Chowdhury, C., & Kamarthi, S. (2021). Evaluation of Human Ear Anatomy and Functionality by Axiomatic Design. *Biomimetics*, *6*(2), 31.

- Sundar, P. S., Chowdhury, C., & Kamarthi, S. (2021). Evaluation of Human Ear Anatomy and Functionality by Axiomatic Design. *Biomimetics*, *6*(2), 31.

- Tekdemir, I., Aslan, A., & Elhan, A. (1998). A clinico-anatomic study of the auricular branch of the vagus nerve and Arnold's ear-cough reflex. *Surgical and Radiologic Anatomy*, *20*(4), 253-257.
- Topsakal, V., Kachlik, D., Carlson, M., Isaacson, B., Broman, J., Tubbs, R. S., & Hans, J. (2021). Relevant temporal bone anatomy for robotic cochlear implantation: An updated terminology combined with anatomical and clinical terms. *Translational Research in Anatomy*, 100138.
- Torigian, D. A., & Ramchandani, P. (2018). Radiology Secrets Plus.
- Uddman, R., Grunditz, T., Larsson, A., & Sundler, F. (1988). Sensory innervation of the ear drum and middle-ear mucosa: retrograde tracing and immunocytochemistry. *Cell and tissue research*, *252*(1), 141-146.
- Umar, M., Chaudhary, S. R., & ur Rehman, M. H. (2017). EXTERNAL EAR. *The Professional Medical Journal*, *24*(05), 778-780.
- Ungar, O. J., Handzel, O., Cavel, O., & Oron, Y. (2019). A smartphone-based weber test may discriminate between a conductive and a sensorineural hearing loss. *Audiology and Neurotology*, *24*(4), 191-196.
- Vayisoğlu, Y., Güven, O., Adanır, S. S., Taghipour, P., Çakır, S., Dağtekin, O., & Talas, D. Ü. (2021). Anatomic features of the fetal round and oval windows, and their relations with the tympanic nerve. *Surgical and Radiologic Anatomy*, 1-19.
- Wahid, F. I., & Nagra, S. R. (2018). Incidence and characteristics of Traumatic Tympanic Membrane perforation. *Pakistan journal of medical sciences*, *34*(5), 1099.
- Walijee, H., Vaughan, C., Munir, N., Youssef, A., & Attlmayr, B. (2021). Microvascular compression of the vestibulocochlear nerve. *European Archives of Oto-Rhino-Laryngology*, 1-7.

CHAPTER 8

APPENDICES

A) BUMDC- FRC Approval letter

Bahria University
Discovering Knowledge
Medical and Dental College, Karachi

Ref no: FRC/BUMDC -13/2020-Ana-112 **MS-11**

Approval of Research Proposal

Mr/Miss/Ms/Mrs/ **Dr. Mariya Azam Khattak**

Registration No:

Dear MS/MPhil Student,

I am pleased to inform you that your research proposal on **"Morphological Variations in Ear Ossicles and Typanic Memrane with age in Correlation with Hearing Thresholds"** has been approved. You may, therefore, continue your research on this theme and produce a quality thesis, as per the HEC requirements.

I take this opportunity to remind you that you must complete your thesis, and defend it successfully, by **SPRING 2021**; this is the date which marks the end of the Extended Duration of your programme. However, to remain eligible for honours and awards, you must complete the thesis, and successfully defend it, by the end of 10 week of final semester.

I wish you every success.

Dated: 28/09/20

CHAIRPERSON FRC, BUMDC

Distribution:

- DG
- Principal
- Student's File (with the HOD/PGP Coordinator)
- Student

B) BUMDC- ERC Approval letter

BAHRIA UNIVERSITY MEDICAL AND DENTAL COLLEGE

Defence phase II, Sailor Street, adjacent to PNS Shifa, Karachi. Tel: 021-35319491-9

ETHICAL REVIEW COMMITTEE

LETTER OF APPROVAL

Date: 15-Jan-21

FRC Reference:
FRC/BUMDC -13/2020-Ana-112

PATRON
Prof. Ambreen Usmani
Principal & Dean
Health Sciences(BU)

CHAIRPERSON
Dr. Quratulain Javaid

SECRETARY
Dr. Ambreen Surti

MEMBERS
Prof M Alamgir
Prof Anis Jafarey
Ms Nighat Huda
Surg Cdre Amir Ejaz
Prof Reza H Syed
Ms Shabina Arif
Mr M Amir Sultan
Surg Lt Cdr Farah
Surg Lt Cdr Sadia

Dr. Mariya Azam Khattak

Subject: Institutional Approval of research study

Title of Study: "Morphological variations in ear ossicles with age in correlation with hearing thresholds"

Principal Investigator: Dr. Mariya Azam Khattak

Reference No: ERC 28/2021

Dear Dr. Mariya Azam Khattak,

Thank you for submitting the above mentioned study proposal. ERC Bahria University Medical and Dental College has reviewed this project in the meeting held on 14-Jan-2021 and gives approval. Kindly notify us when the research is complete.

Regards,

15/1/2021

DR. AMBREEN SURTI
Secretary, ERC
BUMDC

15/1/2021

DR. QURATULAIN JAVAID
Chairperson, ERC
BUMDC

Cc:
DG-BUMDC
Principal BUMDC
Chairperson ERC

C) Consent form (English)

I am giving my consent to participate voluntarily and at my own will in the research project that aims for the morphological variations in ear ossicles with age in correlation with hearing thresholds. The project will evaluate the changes that will help in the future prosthetic and surgical procedures.

I have been explained in detail the nature and significance of participating in the project that morphometric analysis of my sound ear ossicles will help me in reconstructive procedure being performed in my other ear with damaged ossicles that needs to be replaced to improve artistic renderings of the blueprints provided, for prosthetic designs. In any ear pathology the disease can erode the ossicles causing hearing loss providing that if not the record will be entered in my medical history for the future purposes and I understand the provided explanation.

I have been told that all the findings and my personal data will be kept strictly confidential and will be used only for the benefit of otorhinolaryngologist to perform the microsurgical maneuvers and manipulations, so as to correct any abnormalities related to it, surgeons need to be fully conversant about the details of these tiny bones. This study was designed to report about the various variations of the ossicles which would help them while performing the reconstructive surgeries. And will also be strictly only used for the well-being of community, in publications and paper presentations.

I have been explained that radiological investigations will be conducted to evaluate my health status and for this purpose I fully agree.

I also agree to give all relevant information needed, in full and to the best of my knowledge to the researcher. It is clarified to me that no incentive, financial assistance or reimbursement will be provided to me for participating in the study whereas I do have the right to withdraw from the study at any time.

I am advised to contact **Dr. Mariya Azam Khattak** on
Mobile number: **03326798092** or visit **PNS Shifa** hospital in case of query.
Name of Participant: ______________________
S/o, D/o, W/o__________________
Signature of Participant: _________________
Name of Researcher: ____
Signature of Researcher: ______________
Date: _______________

D) Consent form (Urdu)

میں رضاکارانہ طور پر اور اپنی مرضی سے اس تحقیقی منصوبے میں حصہ لینے کے لئے اپنی رضامندی دے رہا ہوں جس کا مقصد کانوں کی ossicles میں شکل کی مختلف حالتوں کو سننے کی دہلیز کے ساتھ ارتباط میں عمر کے ساتھ کرنا چاہتا ہے۔ پروجیکٹ ان تبدیلیوں کا جائزہ لے گا جو مستقبل میں مصنوعی اور جراحی کے طریقہ کار میں مددگار ثابت ہوں گے۔ مجھے منصوبے میں حصہ لینے کی نوعیت اور اہمیت کے ساتھ تفصیل سے بتایا گیا ہے اور میں فراہم کردہ وضاحت کو سمجھتا ہوں۔

مجھے بتایا گیا ہے کہ تمام نتائج اور میرے ذاتی ڈیٹا کو سختی سے خفیہ رکھا جائے گا اور یہ صرف برادری ، اشاعتوں اور کاغذی پریزنٹیشن کے مفاد کے لئے استعمال ہوگا۔

مجھے بتایا گیا ہے کہ میری صحت کی حالت کا اندازہ کرنے کے لئے ریڈیولوجیکل تحقیقات کی جائیں گی۔ اس مقصد کے لئے میں پوری طرح متفق ہوں۔

میں پوری طرح سے اور اپنی جانکاری کی بہترین تحقیق محقق کو دینے کے لئے بھی ضروری ہوں۔ یہ بات مجھ پر واضح کی گئی ہے کہ مطالعے میں حصہ لینے کے لئے مجھے کوئی ترغیبی ، مالی امداد یا معاوضہ فراہم نہیں کیا جائے گا جبکہ مجھے کسی بھی وقت مطالعے سے دستبرداری کا حق حاصل ہے۔

مجھے ڈاکٹر ماریہ اعظم خٹک سے رابطہ کرنے کا مشورہ دیا گیا ہے

موبائل نمبر: 03326798092

یا میری بیماری سے متعلق سوال / ایمرجنسی کی صورت میں پی این ایس شیفا اسپتال ملاحظہ کریں۔

شریک کا نام: ________________________

S / O ،D / O ،W / O ____________________

شریک کے دستخط: ___________________

محقق کا نام: ____

محقق کے دستخط: ______________

E) Questionnaire

S#	DEMOGRAPHICS:
1.	Name:
2.	Gender:
3.	Height:
4.	Age:
5.	Weight:
6.	Ethnicity:
7.	Marital status:
8.	Job description:
9.	Education:
10.	Phone no:

PRESENTING COMPLAIN:

FAMILY HISTORY:

HISTORY OF COMORBITIES:

EAR INVOLVED:

Diseased ear	
Diagnosis of diseased ear	
Normal ear	

ASSESSMENT OF HEARING:

S#	Question	Yes	No
1.	Do you have issue in hearing via phone?		
2.	Improve through one ear than the other when you are on the phone?		
3.	Do you experience difficulty following discussion with at least two individuals talking simultaneously?		
4.	Do individuals complain that you turn the TV volume excessively high?		
5.	Do you need to strain to get discussion?		
6.	Do you experience difficulty hearing in loud sounds?		
7.	Do you have unsteadiness, torment or ringing in ears?		
8.	Do you experience difficulty hearing in eatery?		
9.	Do you discover requesting that individuals repeat themselves?		
10.	Do relatives or associates comment about your missing what has been said?		
11.	Do numerous individuals you talk appear to mutter?		
12.	Do you misjudge what others are stating and react improperly?		
13.	Do you experience difficulty understanding the discourse of lady and youngsters?		
14.	Do people get irritated in light of the fact that you misconstrued what they state?		

CLINICAL HISTORY

S#	Question	Yes	No
1.	Have you at any point had ears inspected to survey hearing? if yes when (date to mentioned)		
2.	Do you experience any problem related to hearing? If yes kindly describe it?		

SURGICAL HISTORY:

S#	Type of surgery	Yes	No
1.	Tympanoplasty		
2.	Ossiculoplasty		
3.	Any other surgery done concerning ear? If yes mention (name of the surgery)		
4.	Surgery other than ear? If yes mention (name of the surgery)		

HISTORY OF ANY TRAUMA

WORKPLACE

1.	How might you depict commotion level at your work site on the basis of level of noise?		
	Zero		
	Low		
	High		
	Extreme high		
2.	Do you feel ringing of the periods toward the day's end	**Yes**	**No**

CLINICAL INVESTIGATION:

S#	Examination	Yes	No
1.	Pure Tone audiometry		
2.	Otoscopic		
3.	Ct scan		

MEASUREMENTS:

PART OF BONE	Reading (mm)
Malleus (width of head)	
Malleus (handle)	
Total length of malleus	
Total width of incus	
Total length of incus	

VARIATIONS (SHAPE)	CURVED	STRAIGHT
Malleus (handle)		

INVESTIGATION PERFOMED:

Name of investigation:	
Date of investigation performed:	
Patients ID:	

F) Plagiarism report

THESIS

ORIGINALITY REPORT

6%	5%	2%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	qdoc.tips Internet Source	3%
2	www.ncbi.nlm.nih.gov Internet Source	1 %
3	Hinrichsen, . "THE EAR", Organ Histology A Student s Guide, 1997. Publication	<1 %
4	Cutaneous Flaps in Head and Neck Reconstruction, 2014. Publication	<1 %
5	Isabel Lockard. "Desk Reference for Neuroanatomy", Springer Nature, 1977 Publication	<1 %
6	John Philip Patten. "Neurological Differential Diagnosis", Springer Science and Business Media LLC, 1996 Publication	<1 %