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THYROID GLAND

MORPHOMETRIC AND HISTOLOGICAL ANALYSIS

DR. BHEEMSHETTY S. PATIL
DR. PALLAVI S. KANTHE

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1 INTRODUCTION

Thyroid meaning – thyreos – an oblong shield; eidos – form in 1656, the anatomist Thomas Wharton named the gland 'Thyroid' because of its shape resembling like a shield.

In 1000 BC ancient sage and father of surgery 'Sushruta' dissected the body found the Thyroid gland, then he coated it as GALA GRANTHI. He is the first ancient surgeon who operated & did the thyroidectomy by preserving all vessels and nerves. And he named the goiter as GANDAMALA. Since 1600 BC the Chinese were using burnt sponge and seaweed for the treatment of goiter (enlarged thyroid gland).

Dysfunction and anatomic abnormalities of thyroid are among the most common diseases of the endocrine glands.

Goiter is one of the common diseases in our country. Due to this fact the thyroid became an organ of interest of research workers.

In human beings, the thyroid gland is one of the largest of the endocrine organs. It is one of the earliest endocrine organs to differentiate and has an important hormonal role in embryonic development. The important function of thyroid hormones is that it maintains the level of metabolism in almost all the body cells that is optimal for their normal function. Thyroid hormones stimulate the oxygen consumption of most of the cells in the body, help to regulate lipid and carbohydrate metabolism and are necessary for normal growth and maturation.

The consequence of thyroid gland is to promote growth and development of the brain and whole body during fetal life and for the first few years of postnatal life. Iodine deficiency is the primary and preliminary single most common cause of preventable mental retardation and brain damage in the world. It causes and leads to enlargement of thyroid (goiter) and reduces the production of hormones vital to growth and development.

The thyroid gland is not essential for life, but its absence causes mental and physical slowing, poor resistance to cold and in children mental retardation and dwarfism.

Thyroid gland is essential for normal growth during prenatal period. Thyroid has been extensively studied in animal and human fetuses, over the years. Yet many details regarding microscopic structure of thyroid during different stages of development in prenatal period or till not very clear.

Hence in the present study an attempt is made to study the histogenesis of thyroid at different stages of development in intrauterine life.

ANATOMY OF THYROID GLAND

The thyroid gland is one of the largest, butterfly shaped, highly vascular endocrine gland situated below the larynx, in front of and sides of trachea opposite C5 to T1 vertebrae. It consists of two symmetrical lobes united by isthmus that lies in front of 2^{nd} to 4^{th} tracheal rings. It weighs about 25gms, slightly heavier in females. In addition to its own capsule, the gland is enveloped by a sheath derived from the pretracheal layer of deep cervical fascia, which sends septa conveying a rich blood supply together with lymphatics and nerves.

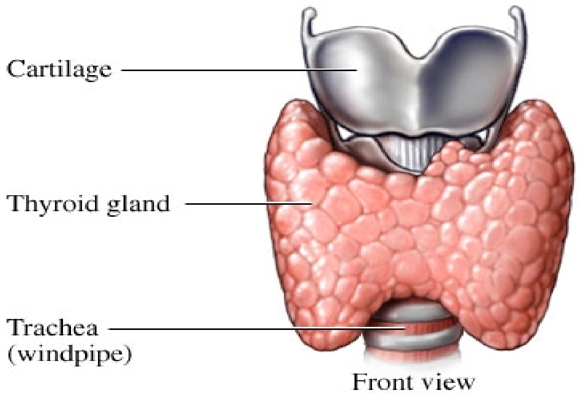

Fig 1 Gross structure of Thyroid Gland and its location

Each lobe of thyroid extends from the oblique line of the thyroid cartilage to the sixth tracheal ring, with its lateral surface covered by infrahyoid muscles. Medial surface lies against the larynx, trachea, the pharynx and oesophagus along with the cricothyroid muscle and its external laryngeal nerve. In the groove between trachea and oesophagus is the recurrent laryngeal nerve. The posterior surface overlaps the carotid sheath with its content. Along its posterior border lie the superior and inferior parathyroid gland and anastomosis between superior and inferior thyroid arteries.

BLOOD SUPPLY

It is a highly vascular gland, having a blood flow about five times the weight of gland each minute (4 to 6ml/gm). The arteries supplying the gland are superior and inferior thyroid arteries which are branches from external carotid and thyrocervical trunk respectively, sometimes the thyroidea ima branch of arch of aorta.

The right lobe of the thyroid is normally more vascular then left, and is often the larger of the two. The arteries ramify in the stroma of the gland as dense, fenestrated capillary plexus, which surrounds the follicles and are accompanied by lymph capillaries. A number of lobular arteries passing in the interlobular fibrous septa supply a group of 40 to 60 follicles. The veins form a plexus on its surface deep to the true capsule. From this three veins arise, superior, middle and inferior thyroid. Superior and middle drain into internal jugular vein and inferior in to left brachiocephalic vein.

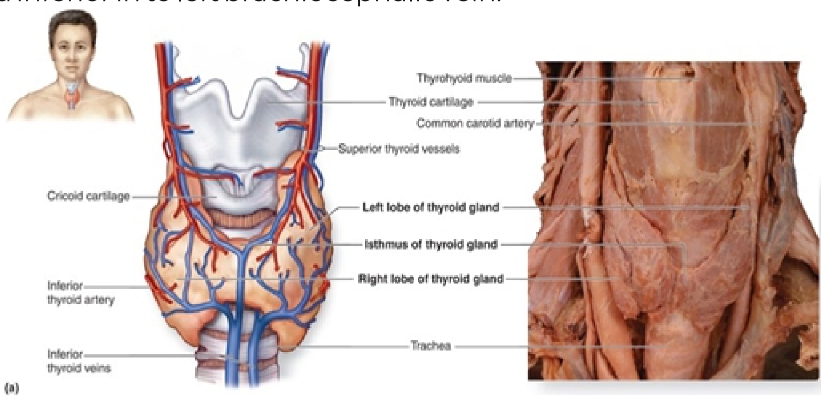

Fig 2 Gross structure of Thyroid Gland and its blood supply

The lymphatics from thyroid drains mainly in to the deep cervical lymph nodes and it is innervated by postganglionic sympathetic fibers from the superior, middle and inferior cervical ganglia.

EMBRYOLOGY: Thyroid tissue is confined to and is present in all vertebrates, even as far back as in phylogeny, as chordates. Though no recognizable thyroid tissue is present in invertebrate species, monoiodtyrosine and diiodotyrosine are present in these species. In **amphioxus** the endostyle lying in the floor of pharynx is concerned with iodine uptake and the synthesis of iodinated compounds, while the same organ in **ammocoetes** undergoes structural changes during metamorphosis, in the course of which it breaks away from the gut.

In the lamprey the structure persists as a series of vesicles having the characteristic structure of thyroid tissue. In most **fishes and reptiles** the thyroid exists as a single structure but the gland becomes bilobed in some **lizards and in mammals** and is then usually connected across a midline by an isthmus.

Thyroid is one of the earliest forming and functioning endocrine organs within the human embryo. The human thyroid enlarge, is first recognizable about 24 days after fertilization (at 20 somite stage) when the embryo is approximately 3.5 to 4.0mm in length. The thyroid primordium begins as a median endodermal thickening in the floor of primordial pharynx. It soon forms a bi-lobed diverticulum attached to the buccal cavity by a stalk – the thyroglossal duct. With development, the duct elongates and the developing gland descends in the neck. The hollow thyroid primordium soon becomes solid cords and bilobed nature of its glandular swelling becomes more marked.

By the 7th week, it comes in contact with caudal pharyngeal complex. The cells of this disseminate within it, giving rise to Para follicular cells of thyroid gland called 'C' cells. Para follicular cells differentiate from neural crest cells that migrate from pharyngeal pouches. During this week only, the thyroid assumes its definitive shape, thyroglossal duct disappears and thyroid reaches its final site in the neck.

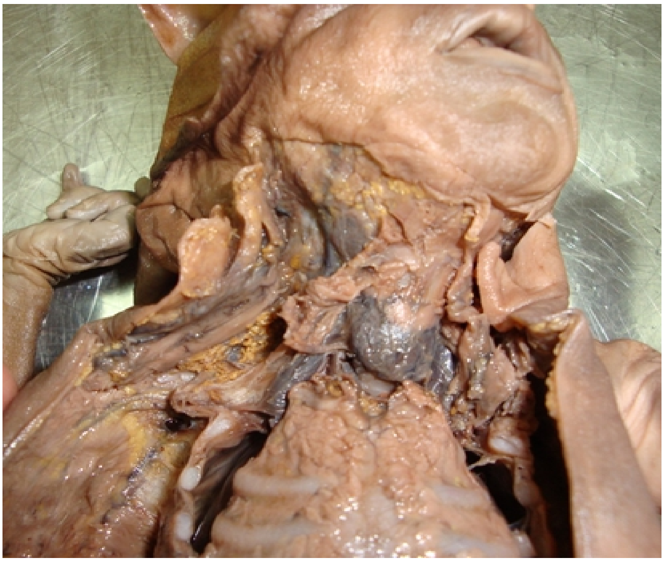

Fig 3 Gross structure of Thyroid Gland and its location in full-term stillborn baby

HISTOGENESIS: The human thyroid does not develop synchronously throughout, but still the maturation of colloid containing follicles is divided in to 3 periods.

1) The precolloid stage – (22-65 mm CRL) – 7 to 13 weeks.
2) The colloid formation stage – (65-80 mm CRL) – 13 to 14 wks.
3) The follicular stage – (after 80 mm CRL) – 14 wks onwards.

The endodermal cells of thyroid primordium multiply and form solid cords. By 10th wk, the cords divide into cellular groups. Soon a lumen forms in this cluster and the cells get arranged in a single layer around a lumen. Complex interconnecting cord like arrangement of the cells interspersed with vascular connective tissue, replace the solid epithelial mass and become a tubule like structure at about 12th wk of fetal life. Shortly thereafter follicular arrangement, devoid of colloid appears. During 13th wks colloid appears in these arrangements. Thereafter iodine concentration and the synthesis of thyroid hormones begin. During the final follicular phase of development, colloid spaces increase in size and there is progressive cell growth and accumulation of thyroid hormones. At 12th weeks of gestation thyroid gland weighs about 80mg and at term it weighs 1 to 1.5 gm.

At birth the cells lining the follicles are composed of cuboidal, with small central nuclei surrounded by a moderate amount of colorless cytoplasm. The cytoplasm often bulges into the lumen of the gland, producing a scalloped margin for the colloid. The largest follicles are located at the periphery of the lobules.

HISTOLOGY: The thyroid is unique in having a histological organization that provides for extra cellular storage of its product in the lumen of cyst like follicles.

Two capsules outer and inner cover the gland. Inner one sends septae into gland carrying blood vessels, lymphatic and nerves. But as septae are incomplete, the gland is not truly lobulated but pseudolobulated.

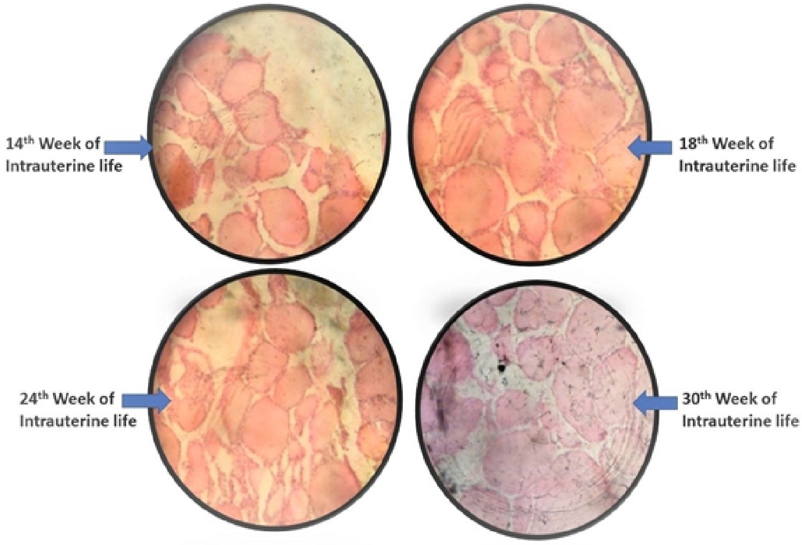

Fig 4 Histological architecture of Thyroid Gland at different level of gestation

The functional and structural units of thyroid gland are thyroid follicles. In human the follicles are estimated to number (2-3) x 10^7 and vary from being rounded to tubular in shape, ranging from 0.02 to 0.9mm in diameter. The follicles are bounded by simple low cuboidal epithelium, polarizing towards the lumen, which is filled with gelatinous homogenous viscous fluid called as colloid. The hormones thyroxin and triidothyronine are stored in the colloid as secretory glycoproteins – which combine with iodine to form thyroglobulin. After fixation, it stains with eosin and appears as solid, structureless, acidophilic pink material. Because of its Carbohydrate content it stains very well with PAS. (Periodic Acid Schiff reaction). A thin basal lamina, a delicate network of reticular fibers and a plexus of capillaries envelop each follicle.

Cell types in Follicles: - The epithelium of thyroid follicles contain two cell types – Principal cells (proper follicular cells) that make up the greater part of the epithelium and Para follicular cells – that occur singly or in small groups between the bases of the principal cells. The epithelium is usually low cuboidal but varies somewhat in height from follicle to follicle and in different state of physiological activity. In quiescent glands – squamous or cuboidal. In hyperactive glands - columnar. But functional activity cannot be based on the height of the epithelium alone.

The follicular cells have round or ovoid nucleus, poor in heterochromatin and containing one or two nucleoli. The cytoplasm of the cell is basophilic. The apical cytoplasm contains numerous dense granules – positive for acid hydrolase which are lysosomes. Also vacuoles with content that stain with aniline blue and with PAS reaction are observed in apical cytoplasm.

With electron microscopy, the thyroid follicular epithelium has many features in common with other secretory cells and some peculiar to the thyroid. From the apex of the follicular cell, numerous microvilli extend into the colloid. It is at or near this surface of the cell that iodination, exocytosis, and the initial phase of hormone secretion occur. The nucleus has no distinctive features and the cytoplasm contains an extensive endoplasmic reticulum (ER) laden with microsomes.

ER is composed of a network of wide, irregular tubules that contain the precursor of Tg. The carbohydrate component of Tg is added to this precursor in the golgi apparatus, which is located apically. Lysosomes and mitochondria are scattered throughout the cytoplasm. Stimulation by TSH results in enlargement of golgi, formation of pseudopodia at the apical surface and the appearance in the apical portion of cell of many. Droplets that contain colloid taken up from the follicular lumen.

The thyroid also contains Para follicular cells which lies singly or in groups and rest either among follicular epithelium or in the thyroid interstitium. They differ from follicular epithelium in never bordering on the follicular lumen and in being rich in mitochondria.

FUNCTIONS OF THYROID GLAND

Thyroid gland produces hormones of 2 types. A) Iodine containing hormones tri-iodothyronine (T_3) and thyroxin (T_4) Calcitonin.

1. T_3 is more potent than T_4. Thyroid hormone regulates the basal metabolic rate and has an important influence on growth and maturation, particularly the nervous tissue.
2. Calcitonin – this hormone regulates blood calcium levels in conjunction with parathyroid hormone. Calcitonin lowers blood calcium level by inhibiting the rate of decalcification of bone by osteoclastic resorption and by stimulating osteoblastic activity.
3. Thyroxin increases the transcription of large number of genes – so that synthesis of protein enzymes, structural proteins, transport proteins enzymes, structural proteins, transport proteins increases greatly i.e. generalized increase in functional activity of cell.
4. It increases the number and activity of mitochondria.

2 HISTORY AND REVIEW OF LITERATURE

Historical references to what we now know as the thyroid gland arise early in medical history.

In 1000 BC ancient sage and father of surgery- 'Sushruta' dissected the body and found the Thyroid gland, then he coated it as GALA GRANTHI. He is the first ancient surgeon who operated & did the thyroidectomy by preserving all vessels and nerves. And he named the goiter as GANDAMALA.

In 1600 BC the Chinese were using burnt sponge and seaweed for the treatment of goitres. In 15 AD, Pliny referred to epidemics of goitre in the Alps and also mentioned the use of burnt seaweed in it's treatment. In 150 AD, Galen, an instrumental figure in the transition from ancient to modern medicine, referred to 'spongia usta' for treatment of goitre.

In 650 AD, Sun Ssv-Mo used a combination of seaweed, dried powdered mollus shells and chopped up thyroid gland for the Treatment of goitre. Ali-inb-Abbas was the first to discuss surgery as a treatment for goitre in 990 AD. Jurjani's 'Treasure of Medicine' in 1110 AD first associated exophthalmus, the protrusion of the eyes we now associate with graves disease, with goitre. In 1475, Wang Hei described anatomy of the thyroid gland and recommended that the treatment of goitre should be dried thyroid. After that Paracelsus, attributed goitre to mineral impurities in the water.

Finally, in 1656 Thomas Wharton named the gland 'thyroid', meaning a shield. But still in 1656, it was thought that the main function of the thyroid gland was to lubricate the trachea. It was also believed to have a cosmetic function in women. Paintings going back to ancient Egypt often emphasize the size of the thyroid gland in women. In the early 1800s, the thyroid was thought to be a vascular shunt to divert the blood flow from the brain. In 1811, cancer of the thyroid was the first disease of the thyroid to be described.

Rush reported in 1820, that the thyroid gland is longer in women because it is "necessary to guard the female system from the influence of the more numerous causes of irritation and vexation of mind to which they are exposed than the male Sex." Also in 1820, Hofrichter stated: "If it were indeed true that the thyroid contains more blood at some times than at other, this effect would be visible to naked eye; in this case women would certainly have long ceased to go about with bare necks, for husbands would have learned to recognize the swelling of this gland as a danger signal of threatening trouble from their better halves."

In 1825, Parry published the association between an enlarged thyroid gland and the characteristic clinical features of hyperthyroidism. This publication was followed by the classic descriptions of Graves and von basedow in 1835 and 1840 respectively.

Cretinism was described in 1871. In 1874, Gull noted the clinical changes associated with atrophy of the thyroid gland and in 1878, Ord coined the term myxedema because he felt that excessive mucus formation and deposition under the skin were responsible for the characteristic thickening of the subcutaneous tissue.

In 1884, thyroidectomies were successfully performed for the treatment of toxic goitre. In Europe, Theodorn kocher (1841-1917) performed over 2000 thyroidectomies. In 1891, Murray obtained a good clinical response in patients with hypothyroidism by injecting them with thyroid extracts.

Gley, in 1891, was able to differentiate the functions of the thyroid from those of the parathyroid glands. In 1895, Magnus – Levy established the effect of the thyroid on the metabolic rate, the low metabolic rate in patients with hypothyroidism and the fact that the administration of thyroid extracts, to these patients and to individuals with normal thyroid function increased their oxygen consumption. Hashimoto's disease was described in 1912.

Edward kendall, professor of physiological chemistry at the Mayo, isolated thyroxin in crystalline form in 1915, and established that the crystalline form had the same effects as the thyroid extract from which it was obtained. In 1926, Harrington defined the chemical formula of thyroxin, and a year later synthesized the hormone. Triiodothyronine (T3) was synthesized in the early 1950s.

The presence of thyroid Stimulating antibodies in Graves disease was determined in 1956 and that of thyroid antibodies in Hashimoto's disease in 1957. Medullary thyroid carcinoma was recognized as a distinct entity in 1959.

In 1961, calcitonin hormone of thyroid gland was discovered which has a role in calcium metabolism. In 1967, the first description of resistance to thyroid hormone was made. In 1970, it was found that T3 is largely derived from peripheral thyroxin (T4). The T3 – binding receptors were identified in 1972, their homology to the viral oncogener A, was recognized in 1986.

The gene for the B – sub unit of thyroid – stimulating hormone (TSH) was cloned in 1988 and that of TSH receptor in 1989. Point mutations in the thyroid – hormone receptor accounted for hormone resistance which was established in 1989 and 1990.

Many research workers have studied development and histogenesis of human as well as animal thyroid gland during intrauterine period. E.L. Potter (1961) has given the extensive data of body weight and organ weight in different periods of gestation. They have suggested the definite correlation between body weight and organ weight. Their study showed that the thyroid weight increases from 0.8 gm to 2.4 gm from 24th wk to 40th wk of gestational age respectively.

He reported that the thyroid gland originates during the 3rd week as a diverticulum from the floor of the pharynx. It rapidly becomes bilobed and descends in the neck by elongation of the tissue at its point of origin. This stalk known as thyroglossal duct. Initially the gland consists of solid cords of cells. These are gradually rearranged to form small follicles; at first solid, they later become hollowed out and filled with an acidophilic material known as colloid. At birth the cells lining the acini are composed of small central nuclei surrounded by a moderate amount of colourless cytoplasm. The cytoplasm often bulges in to the lumen of the glands, producing a scalloped margin for the colloid. The largest acini are located at the periphery of the lobules. The gland is highly vascular and large numbers of small vessels are present between the acini.

Ahmed S. El – Sheikh, A.A.Rasheed (1966) had extensively studied the thyroid gland in 94 camel fetuses. According to them, epithelial cords appeared in the thyroid at the end of first month of intrauterine life (gestational period of camel is between 12 to 12.5 months). Follicles were first seen in the thyroids from fetuses 45 days old: colloid was present in follicles of fetuses 52 days old, secretory activity begins in the fetal thyroid of the camel about the 7^{th} week and peaks during 8^{th} month while excretory activity begins towards the end of 8th of gestation.

Hamilton and Moss Man (1972) states that, the relative weight of the thyroid gland gradually increases until the fourth month (80 mm CRL). At this time the thyroid first develops the ability to concentrate iodine. After the fourth month the thyroid maintains an equal growth with the body. After the 12^{th} week, the thyroid concentrates iodine more readily. Shortly afterwards oxidation of iodine occurs; subsequently mono-iodotyrosine and then di-iodotyrosine are formed. Finally by midgestation the fetal thyroid is able to couple iodotyrosines, thus completing the synthesis of its own hormones.

Physiologically, there are two stages of thyroid development. During the first, the thyroid is not sensitive to thyroid – stimulating hormone, but during the second, after colloid appears, the thyroid products control the production of thyroid – stimulating hormone (TSH) by a feed – back mechanism via the hypothalamic – pituitary centers. Neither maternal nor fetal TSH crosses the placenta, TSH from the fetal pituitary is probably not necessary for the gross morphological development of the thyroid gland, as evidenced by a thyroid of normal size in anencephalic monsters. But pituitary is necessary for the formation of follicles and colloid: The earlier stage at which thyroid hormone has been found I in a 142mm C.R. length fetus (approximately 135 days).

Aleshin BV, Brindak OI (1983) had studied sex differences in the development of the thyroid gland in human prenatal ontogeny. According to them sexual differences were noted in the appearance of follicles, connective tissue, nerve fibers in the thyroid gland. All these events occurred earlier in the male fetuses. The process of organ differentiation in male fetuses was more active than in the female ones during the whole prenatal period of development. A suggestion is put forward that the sexual hormones of embryonic testicles influence the thyroid gland differentiation.

Maredd (1989) has studied the developmental relations between the weights of internal organs and somatic features like CR length, weight etc. of fetuses and newborns. Weights various organs including thyroid were compared with CR Length and weight of fetuses between 17th to 45th week of age. The analysis of results indicated that there is a statistical developmental relation between the weight of internal organs and the somatic features in the fetal period. The developmental relations are most strongly marked between the 6th and 10th month of life and in post mature fetuses and newborns they are weaker.

Bocian – sobkowska. J. Malendowicz LK, Woznaik W. (1992) carried on morphometric studies on the development of human thyroid gland in early fetal life. They had performed histological and morphometric studies on 30 thyroid glands obtained from normal human fetuses. Their crown – rump length (CRL) ranged from 57 to 190mm, corresponding to the gestational age of 1-20 weeks. The weight of the thyroid gland increased proportionally in relation to CRL, foot length and fetus weight. The first follicles containing the PAS-positive colloid were observed in the peripheral part of the thyroid gland of 57mm CRL fetus.

The number of follicles increased up to 85mm CRL and thereafter gradually declined for the end of studied period of fetal life. The volumes of thyroid epithelium, colloid and stroma, beginning from 85mm increased proportionally to the CRL while the height of epithelial cells did not change. The epithelium / colloid ratio decreased notably to 165mm CRL and thereafter remained constant. Results of the present study suggest that the thyroid gland of human fetus approaches structural maturity in 17.5 weeks of gestation. Stepanov SA, Kirichenko AK, Aleskeev IA (1993) had studied on chronic placental insufficiency and histogenesis of fetal thyroid in late spontaneous abortions. The role of the chronic placental insufficiency in the ontogenetic maturation of fetal thyroid gland was studied in 75 cases. In case of chronic placental insufficiency depression of placental metabolic processes was found associated with disco-ordination of histogenesis of fetal thyroid gland, a decrease of specific capacity of its parenchyma and vessels, as well as with growth of stroma component value.

Takizawa T, yamamoto M, Arishima K, Kusanagi M, Somiya H, Eguchi Y. (1993) had studied an electron microscopic study on Follicular formation and TSH sensitivity of the fetal rat thyroid gland in organ culture.

They have observed that thyroid of 15 days rat fetuses, prior to culture had no follicles. After 2 days in culture, regardless of added TSH in the medium, thyroids had small, colloid – storing follicles. In the follicular cells, the rER and Golgi apparatus were somewhat well developed as compared with the thyroids prior to culture. Thyroids of 17 – day fetuses prior to culture were first inlaid with follicles with TSH, 2 days cultures of 17-day thyroids showed a more marked dilatation of rER and a further development of Golgi apparatus than those without TSH. These results indicate electron microscopically that, TSH has no effect on the first formation of follicles as well as on the initial development of follicular cells, but that TSH can promote the further development of follicular cells themselves of once developed follicles.

Luecke, Wosilait, Young (1995) have stated the mathematical representation of organ growth in human fetus. According to them, total growth is the summation of various organs. In their study, the quantitative representation of correlation of fetal organ weight with total fetal weight was described. The data of analysis have taken from 16 fetuses ranging from 25 to 300 days post conception CR length; CH length, weight of the fetus and weight of various organs including the thyroid were compared with each other.

Bocian – sobkowska J, Woznaik W, Malendowicz LK. (1997) had done morphometric studies on the development of the human thyroid gland in the late fetal life.

They had performed histological and morphometric studies on 27 thyroid glands obtained from normal human fetuses between 23^{rd} 40 wks of intrauterine life. They calculated the volume of gland, colloid, and stroma and height of the thyroid follicular cells. Depending upon epithelium/colloid ration, they described three stages of thyroid development. The first one between week's 10^{th} – 18^{th} is characterized by massive folliculogenesis and gradual accumulation of colloid.

The second stage, between weeks 19^{th} – 29^{th} is characterized by rather unchanged values of epithelium/colloid ratio and the size of follicles. The third stage, after the 29^{th} week of development is characterized by a gradual increase in the epithelium/colloid ratio and a decrease in the size of follicles.

Meinel K, Doring K. (1997) had studied on the growth of fetal thyroid gland in the 2^{nd} half of pregnancy – by biometric ultrasound studies. They studied 21 pregnant women (completed 20^{th} to 41^{st} week for pregnancy). The thyroid was measured by 5 parameters, Rt lobe transverse and P.A. diameter, left lobe transverse and P.A. diameter, Right and Left lobe transverse including trachea. Result was the transverse diameter of both thyroid lobes including the trachea grows from 10mm (20^{th} wk) to 20mm (at 40^{th} wk). The transverse and P.A. diameter of either lobe double in the 2nd half of pregnancy.

Hoss, Metreweli C. (1988) studied, 289 normal fetal Thyroid between 20 wks and 36 wks of gestation. They calculated the fetal thyroid volume by the ellipsoid equation. They showed that the ratio of fetal thyroid volume to gestational fetal weight was constant throughout gestation. The fetal thyroid increased in size much faster after 32 weeks.

Ranzini Ac, Ananth CV. et. Al. (2001) had studied 200 fetuses between 16^{th} – 37^{th} wk for ultrasonography of the fetal thyroid:

nomograms based on biparietal diameter and gestational age, Nomograms of fetal thyroid seize were created by using 5^{th}, 10t, 50^{th}, 90^{th} and 95^{th} percentiles based on biparietal diameter and gestational age. They concluded that both biparietal diameter and gestational age serve as good predictors of fetal thyroid circumference, when the biparietal diameter is difficult to measure, gestational age can be used to assess thyroid size. Radaelli T, Cetin I, Zamperini P. et al (2002) also had studied intrauterine growth of normal thyroid by ultrasonography. They concluded that the fetal thyroid grows between 12 and 39 weeks of gestation with a steepest increase during the second trimester, that is when the fetal thyroid becomes functionally active.

P. Larson, Kronenberg. et al (2003, 10th Edn.) they stated that the human thyroid enlarge is first recognizable about 1 month after conception, when embryo is approximately 3.5 to 4.0mm in length. The primordium begins as a thickening of epithelium in the pharyngeal floor, which forms a diverticulum and is displaced caudally with elongation of stalk, which is called thyroglossal duct. During its displacement, the primordium assumes a bilobate shape, coming into contact and fusing with the ventral aspect of the 4^{th} pharyngeal pouch.

Normally, the thyroglossal duct undergoes dissolution and fragmentation by about the 2^{nd} month. Cells of lower portion of the duct differentiate in to thyroid tissue, forming the pyramidal lobe of the gland, concomitantly histological alterations occur throughout the gland. Complex interconnecting cord-like arrangements of cells interspersed with vascular connective tissue replace the solid epithelial mass and become tubule-like structures at about the third month of fetal life. Shortly thereafter follicular arrangement devoid of colloid appear and eventually the follicles fill with colloid by 70 days of gestation. Fetal thyroid function begins at about the end of first trimester.

Thomos H. Shepard, Henning J. Anderson, Helge (2005) they had studied the human fetal thyroid: The weights of 75 human fetal thyroids were analyzed in relation to crown rump length, foot length, body weight and estimated gestational age. They showed that the relative weights of the thyroid gland gradually increase until fetuses attain a crown – rump length of 80mm. This length represents an age of 80 days gestation and a developmental period, which is functionally and histologically very significant. This period (75-85) days of gestation are when the human thyroid first develops the ability to concentrate iodine. Although small amounts of colloid are present earlier there is also a very sharp increase in amount between 75 and 85 days. After the 80mm period the thyroid weight averages 0.0458% of body weight and this average is close to that of the newborn (0.049, Potter, 61) and adult (0.036).

Along with the development and histogenesis of thyroid other endocrine glands also develop simultaneously, and they also have studied by many research workers.

This period (75-85) days of gestation is when the human thyroid first develops the ability to concentrate iodine. After 80mm period the thyroid weight averages 0.0458% of body weight and this average is close to that of the newborn (0.049, Potter, 61) and adult (0.036). Thus the average percentage relative weight of thyroid in our study, 0.0455 was very similar to the finding quoted by the above mentioned authors.

In the present study, it was observed that thyroid gland increased proportionally in relation to CRL, body weight and estimated gestational age. This observation was very similar to reported by Bocoin – Sobkowska J, Malen Dowicz LK (1992 and 1997) and by Thomas Shepard and Hening J. Anderson (2005).

Development of Histological structure of thyroid is compared with the findings of other workers. According to Potter (1961), during development of human thyroid, the solid epithelial cords gradually rearranged to form small follicles, at first solid which later become hollowed out and filled with an acidophilic material known as colloid. At birth the cells lining the follicle contain of small central nuclei surrounded by a moderate amount of colourless cytoplasm. The cytoplasm often bulges into the lumen of the glands, producing a scalloped margin for the colloid. The largest follicles are located at the periphery of lobules. The gland is highly vascular and large number of small vessels is present between the follicles.

Hamilton (1972) divided the maturation of thyroid follicles into 3 stages.

a) The precolloid stage – (22-65mm CRL) – 7 to 13 weeks.
b) The colloid formation stage – (65-80mm CRL) – 13 to 14 weeks.
c) The follicular stage (after 80mm CRL) – 14 weeks on wards.

The endodermal cells of thyroid primordium become arranged in cord and stands. As early as the 7th week, the thyroid cell contains an intracellular canaliculus, which is lined by microvilli and contains a thin granular substance.

With maturation, colloid formation takes place in the lumen of follicles. During this 2nd stage, there is an increase of blood vessels between the follicles. During the 3rd stage there is a progressive

3 DISCUSSION

Human fetal growth is the net result of a complex interplay of genetic, hormonal and growth factor effects. During intrauterine development of fetus, the thyroid gland is very essential for normal maturation of central nervous system. Congenitally hypothyroid infants with marked hypothyroxinemia may manifest prolonged jaundice, lethargy, feeding difficulties, umbilical hernia, but the classical symptoms are metabolic derangements, growth retardation and irreversible mental and neurological dysfunction.

In the present study, while studying the development of thyroid in antenatal period, the different morphological and histological parameters of thyroid glands were considered.

They are...

1. The study of weight of thyroid gland at different stages of gestation and their proportion to the body weight and crown-rump.
2. The microscopic structures of thyroid at different stages in prenatal period were observed. Points noted were 1) Capsular development & vascularity 2) Appearance of developing follicles 3) Appearance of colloid 4) epithelium of follicles.
3. The diameter of follicles was measured with the help of micrometre scale and micrometre eye piece.

In the present study body weight of fetuses showed gradual increase from 12th week to 38th week of gestation. These findings were compared with the findings of other workers. From the above table the body weight reported by Arey (1934) and Schulz (1962), which was up to 20th week and Potter & Craig (1976), which was 26th week were found to be less than the present study. Thereafter it was more or less comparable with them and Gruenwald (1960).

At the same time, when compared with Hamilton (1960), it was greater up to 28th week and comparable thereafter up to 38th week. However the findings of present study were more or less similar to the findings reported by Parulekar (1995). In the present study the crown-rump length showed gradual increases as the gestational age of fetus increased. These findings were compared with the findings of other workers. The crown-rump length reported by the Moore, Hamilton and Potter was more or less comparable to the present study.

The findings reported by Schulz were found to be greater than the findings of present study. In the present study it was observed that the weight of thyroid gland showed gradual increases with increase in gestational age of fetus (table 7). E.L. Potter had reported the absolute weight of thyroid gland from 24^{th} to 30^{th} week with gradual increase thereafter up to 38^{th} week. Reported weight at 38^{th} week was 1.3 gms. Our study also showed more or less constant weight from 24^{th} to 30^{th} week and gradual increase thereafter. In our study the weight of thyroid gland at 38th week was 1.25 gms.

Williams (2003, 10^{th} edition) has mentioned the weight of thyroid gland as 80mg at 12^{th} week and 1 to 1.5 gm at term, which coincides closely with our study. In the present study, the relative weight of thyroid gland was calculated from 12^{th} week to 38^{th} week of gestation, but the details findings from were not available from previous studies for comparison.

At 12^{th} week of gestation, the percentage relative weight of thyroid was 0.066, which was remained more or less same up to 26^{th} week, at 28^{th} week, percentage relative weight was 0.042 which remained almost constant up to 38^{th} week of gestational age. Nearing full term, the average percentage relative weight was 0.0445.

According to Hamilton (1978), the relative weight of the thyroid gland gradually increases until the fourth month (80mm CRL). At this time the thyroid first develops the ability to concentrate iodine. After the fourth month the thyroid maintains an equal growth with body.

Thomas H. Shepard, Hening J. Anderson, Helge (2005) showed that the relative weights of the thyroid gland gradually increases until fetuses attain a crown-rump length of 80mm. This length represents an age of 80 days gestation and a developmental period which is functionally and histologically very significant.

showed negative reaction. The differentiation of thyroid follicles started from the periphery of the gland and extended centrally, as the periphery of the gland was more vascular than the center throughout the gestational age of fetus.

This observation was similar to the observation reported by Potter (1961) in human thyroid and Ahmed S. et al (1966) in fetal thyroid of the dromedary (camelus dromedarius).

The differentiation of thyroid follicles was prominently seen up to 20-24th week stage of fetal thyroid. As the gestational age advances, the gland showed very little differentiation between centrally and peripherally placed follicles.

The follicles were round, oval or irregular in shape and were of different sizes. As the gestational age advanced, the number of developing thyroid follicles increased. This folliculogenesis was more prominent between 14th – 20th week stage of fetal thyroid. Hamilton (1972) noted the follicular stage at 14th week of gestation or later. Our study correlates with his findings.

The colloid containing follicles with tall cuboidal epithelium were first observed at 13th week stage of developing thyroid. From 14th week, gradual increase in accumulation of colloid with increase in intrafollicular vacuoles was observed with maximum at 20th – 24th week stage of thyroid. At this stage the epithelial cells of follicles were cuboidal with apical position of nuclei and vascular network also abundant. Mature thyroid follicles were present in the fetuses of this group.

The presence of colloid in the follicles, its affinity for acidic dyes, clear vacuoles in the colloid and the apical position of epithelial cell nuclei are considered indicative of the secretory activity of the follicular cells (Mitskavitch, 1957). Our findings correlate with these findings. This indicates that secretory activity of the gland was, more at this stage (20th – 24th week). Bocian – Sobkowska J. et al (1997) showed that the stage of massive folliculogenesis and gradual accumulation of colloid appeared between 10-18th week stages of developing thyroid. Our findings were very much similar as that of above study.

gradual increase in the diameter of follicles. Bocian – Sobkowska J et al (1992 and 1997) have observed that the first follicles containing PAS – positive colloid were observed in the peripheral part of the thyroid gland of 57 mm CRL fetus, which corresponds to 10-12 weeks. The number of follicles increased up to 85 mm CRL. The volumes of thyroid epithelium, colloid and stroma, beginning from 85mm increased proportionally to the CRL, while the height of epithelial cells did not change. According to them, the thyroid gland approaches structural maturity at 17.5 weeks of gestational age.

They divided the intra-uterine development of thyroid into 3 distinct stages.

1. Between 10-18 wks – characterized by massive folliculogenesis and gradual accumulation of colloid.
2. Between 19-29 wks – unchanged values of epithelium/colloid ratio and the size of follicles.
3. After 29^{th} week – a gradual increase in the epithelium / colloid ratio and a decrease in size of follicles.

According to Williams (2003 10^{th} edition) during the development of thyroid, complex interconnecting cord-like arrangements of ells interspersed with vascular connective tissue replace the solid epithelial mass and become tubule-like structures at about the third month of fetal life. Shortly thereafter follicular arrangement devoid of colloid appears and eventually the follicles fill with colloid by 70 days of gestation. Fetal thyroid function begins at about the end of first trimester.

In the present study, the development of thyroid from 12^{th} week to 38th weeks of gestational age was studied and observed. At 12^{th} week, the capsule was thin with small number of blood vessels. But as age advances, the capsule became thick and its vascularity also increased. From the capsule thin septae along with blood vessels were seen invading the stroma of gland, but did not give the gland a lobular appearance. This observation was in agreement with the observation of human thyroid studies of Ham and Carmack (1975). At 12th week, the stroma of the gland consisted of most of the epithelial cells in the form of clusters and cords. Very few small follicles were seen at the periphery of gland. The colloid was not observed. PAS staining

Comparing the ratios between embryonic ages when colloid containing follicles first appeared and the length of a gestation period of different species studies, the following figures were obtained 0.34 for human fetus (present study); 0.50 for man (fenger, 1915; Pattern, 1947); 0.4 for Camel (Ahmed S. et al 1966); 0.31 for Bovine (Koneff et al 1949); 0.35 for chicks (Brandway, 1929); 0.65 for rabbits (Aleshen, 1954); 0.88 for mice (chardard Raimboutt, 1949) and 0.93 for rats (Mitskavitch, 1957).

It was observed that presence of first colloid containing follicles occurred much earlier in the camel embryo. This may be associated with the relatively advanced state of body development in camels at birth. Hall and Kaan (1942) observed that the rat embryo differentiates late and that its thyroid differentiates shortly before birth.

In present study, the observed ratio (0.34) was lower than the reported by Fenger and Pattern (0.5). This might be because of ethnic and genetic variation of developing fetuses.

In the present study, from 28th week onwards, the gland showed rich vascularity network surrounding the follicles. In most of the follicles, the colloid appeared irregular and erobed at the periphery alongside the follicular cells. This is commonly observed mainly in active follicles of fixed and stained histological preparation as reported by I.C. Junqueir et al (1975).

Arthur W. Ham (1974) reported that the colloid is often seen to have shrunken away from the follicular epithelium in such a way as to present a serrated rather than a smooth outline, when the gland is active our study correlates with above findings.

The PAS staining was done to confirm the colloid stage of thyroid. In the present study, PAS positive reaction was observed from 13th weeks onwards. The diameter of thyroid follicles at different gestational weeks was measured by the micrometer scale and micrometer eye piece. Size of thyroid follicles in developing fetuses has not been reported so far in details. To measure the diameter, round and oval shaped follicles were taken into consideration. In case of oval shaped follicles the maximum diameter is taken into consideration.

The size of peripheral and central follicles at 12th week was 34-40 μ m and 16-28 μ m respectively. Then it increased slowly up to 38th week of gestation, when the size of centrally placed follicles averages up to 140 μ m and that of the peripheral follicles averages up to 200-240 μm. Thus towards the term, size of thyroid follicles approached closer to that of adult average size i.e. 200 μ m as reported by Williams (2003, 10th edn.).

Thus in the present study, the histological structure of thyroid was studied at different fetal ages, and the development was found to be in accord with earlier studies.

4 SUMMARY

The body weight crown-rump length and thyroid weight of each of the fetus were recorded. The mean value was calculated for different stages of gestation. The relative weight of thyroid was calculated.

Histological structure of thyroid was observed from early to late fetal period – Nature of epithelium, appearance and amount of colloid content and capsular and vascular development was studied in fetuses of different gestational age.

In addition, sizes of thyroid follicle and carbohydrate contents in the colloid of follicles at different weeks of gestation were observed.

The increase in weight of thyroid gland in human fetuses seems to be directly proportional to the

a. Increase in the body weight of fetuses
b. Increase in crown-rump length of fetuses
c. Increase in estimated gestational age

The weight of thyroid at 12th week of gestation was 0.0717 gms. It increased gradually up to 0.517 gms at 28th week of gestation. Thereafter it increased with faster rate. The weight at 38th week gestation was 1.25 gms.

The average percentage relative weight towards term was 0.0445 which was close to that of the newborn (0.049, Potter 61) and adult (0.036).

The microscopic differentiation of thyroid.

a. At 12th week, thin capsule, clusters and cords of epithelial cells with peripheral differentiation of few follicles were seen.
b. First colloid containing follicles were observed at 13th week's stage of thyroid.
c. Folliculogenesis with increased vascularity reached maximum at 14th – 20th week stage of thyroid.

d. Mature thyroid follicles with increased secretory activity were seen at 20th – 24th week stage of thyroid.
e. Nearing full term, adult type mature thyroid follicles were observed.
f. Diameter of thyroid follicles was measured by micrometer scale and micrometer eye piece. It averaged 120-240 μ m in diameter nearing full term which was close to the adult average size i.e. 200 μm.

On PAS staining, colloid showed negative reaction at 12th week and positive reaction at 13th week onward. With the help of above observations, it can be concluded that depending on the microscopic differentiation and organization, developmental staging gat thyroid could be done as following:

- The precolloid stage – up to 12th week.
- Colloid stage – 13th week with max at 20th week.
- Folliculogenesis stage – 14th – 20th week.
- Secretory activity – 20th – 24th week.

This study is helpful for the benefit of General Surgeons, ENT Surgeons and Endocrinologists.

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