ABSTRACT

Background: The importance of thyroid gland is to promote growth and development of the brain during fetal life and for the first few years of postnatal life. Iodine deficiency is the single most common cause of preventable mental retardation and brain damage in the world. Objective: To correlate the weight of fetal thyroid with body weight, crown-rump length, and estimated gestational age of fetus. To study microscopic structure of the human thyroid in gestational age groups of normal, still born fetuses. To correlate the size of thyroid follicles with increasing gestational age. To study the nature and amount of colloid content of the follicles at different stages of development. Results: In the present study it was observed that the weight of thyroid gland showed gradual increases with increase in gestational age of fetus. 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. Conclusion: The increase in weight of thyroid gland in human fetuses seems to be directly proportional to the increase in the body weight of fetuses, increase in crown-rump length of fetuses, increase in estimated gestational age.

INTRODUCTION

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 importance of thyroid gland is to promote growth and development of the brain during fetal life and for the first few years of postnatal life. Iodine deficiency is the single most common cause of preventable mental retardation and brain damage in the world. It causes enlargement of thyroid (goiter) and decreases 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.

MATERIALS AND METHODS

In presence study, 50 still born, normal fetuses (30 male, 20 female) were obtained with the permission of Professor and HOD of obstetrics and Gynecology, MNR Medical College and Hospitals, Sangareddy. These fetuses included the spontaneous abortuses and stillborns. Twins and fetuses with gross anomalies were omitted from the study. Fetuses were obtained within 4-5 hrs. of birth avoid post-mortem changes.
Measurement of external parameters:

The gestational age, sex, weight, crown-rump length were studied in detail. The gestational age determined by last menstrual period and crown rump length ranges from 12th week to 36th week.

Weight of fetuses was measured in grams on digital weighing machine. The crown-rump length was measured by using measurement plastic tape and then scale with using divider.

Fixation of fetuses:

The fixation of the fetuses was ensured by injecting 10% of formalin locally on various sites with the help of 10ml syringe and 20 number needles in cranial cavity, thoracic cavity, in the neck and subcutaneously in the upper and lower limbs.

Dissection, Measurement and Fixation of Thyroid:

Fetuses were carefully dissected. Taking midline incision opened the anterior of the neck. The infrahyoid muscles were separated and the thyroid gland was removed. Then it was weighed immediately and fixed in 10% formalin. The thyroid was cut into pieces and fixed in Bouins medium for 24 hrs. After fixation, it was placed in 70% alcohol for 6-8 hours during day, then in 90% alcohol for overnight. Next day three changes of absolute alcohol were given for one hour each.

The tissue was blotted with blotting paper and placed in xylene for about 30 min. for clearing. Then tissue was subjected to 3 changes of paraffin wax at 560 C - 600 C for one hour each. Then tissue was embedded. ‘L’ shaped moulds were smeared with glycerin and fresh filtered wax poured into it to fill it almost. Any air bubbles formed were removed by hot spatula. The tissue was fixed on one side of mould and label was placed on the opposite side of the mould. After a skin of wax has formed completely over the surface of the block, its solidification was hastened by careful immersion in cold water, for 15 min. Then the block was removed from mould.

The blocks were prepared for cutting. Finally tissue was cut at the sizes of 5 to 7 μ in the form of ribbon.

The individual sections were gently lowered on to the surface of water at 50 C to 100 C to remove the folds. The sections were taken on egg albumin coated slides. Slides were kept for drying on a hot plate at 450 c - 500 C for 2 hours or more as per requirement.

Staining:

1. Removal of paraffin wax was done by dipping into xylene one or two min. in each of two changes of xylene.
2. Removal of xylene was done by dipping into two changes of absolute alcohol for one hour to one min. each.
3. Then it was followed by treatment for a minute or two with 90% alcohol and then 70% alcohol.
4. After this, slides were kept under running tap water for about 5 minutes.
5. Then stained in haematoxylin for about 10-15 min and again kept under tap water for 5 min.
6. Excess stain was removed by dipping into acid alcohol for a few seconds. Here the blue color was changed to red because of acid.
7. The blue color ( bluing ) was regained by washing in alkaline, running tap water for 5-10 min. The stain was checked.
Table 1: Showing comparisons of body weight in (gms) of present study with the findings of other workers.

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Table 2: Showing comparisons of crown-rump length (in mm) of present study with the findings of other workers.

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Table 3: Showing the comparison of thyroid gland weight (in gms) of present study with the findings of E.L. Potter.

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<th>Weight of thyroid (in gms)</th>
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<td>1.3</td>
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</table>
In the present study it was observed that the weight of thyroid gland showed gradual increases with increase in gestational age of fetus.

E.L. Potter had reported the absolute weight of thyroid gland from 24th to 30th week with gradual increase thereafter up to 38th week. Reported weight at 38th week was 1.3 gms. Our study also showed more or less constant weight from 24th to 30th week and gradual increase thereafter. In our study, the weight of thyroid gland at 38th week was 1.25 gms.

Williams (2003, 10th edition) has mentioned the weight of thyroid gland as 80mg at 12th 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 12th week to 38th week of gestation, but the details findings from were not available from previous studies for comparison.

At 12th week of gestation, the percentage relative weight of thyroid was 0.066, which was remained more or less same up to 26th week, at 28th week, percentage relative weight was 0.042 which remained almost constant up to 38th 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.

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 Bocian – 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 thecolloid. 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 onwards.

The endodermal cells of thyroid primordium become arranged in cord and stands. As early as the 7th week, the thyroid cell contains an intracellular canalculus, 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 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 29th week – a gradual increase in the epithelium/colloid ratio and a decrease in size of follicles.

According to Williams (2003 10th 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 12th week to 38th weeks of gestational age was studied and observed.

At 12th 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 showed negative reaction.

The differentiation of thyroid follicles started from the periphery of the gland and extended centrally, as the gestational age advances, 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 folliculogenisis 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 stage of week 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 cells in the form of clusters and cords were 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 PAS staining was done to confirm the colloid stage of thyroid. In the present study, PAS positive reaction was observed from 13th week onwards.

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 colloid was studied at different fetal ages, and the development was found to be in accord with earlier studies.

### CONCLUSION:

1. 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 36th week gestation was 1.25 gms.

2. 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).

3. 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 μm 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 of 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.

References


28) Sushrata Samhita Chikitsa Sthana 18th chapter, Sloka Nos. 5 – 12, Page No.172 illustrated Sushrata Samhita by P. B. S. Srikanth Murthy.


