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Original Article

Histological study of thyroid gland among fetus in different age groups

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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.

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cardiovascular cells. Molecular genetics studies reveal that RAS also takes part in the pathogenesis of cardio-cerebrovascular diseases. Consequently angiotensinogen gene (AGT), the only incipient substrate of angiotensin precursor, is widely observed as one of the genes associated to cerebrovascular diseases. The gene that encodes angiotensinogen is found on chromosome 1q42 to 43 where a tyrosine for cytosine substitution in the second exon results in the substitution of threonine for methionine at amino acid position (Met235Thr) in the translated protein. In humans, the Thr235 allele has been reported to elevate the serum level of AGT, causing an increase in blood pressure. In the renin-angiotensin system, AGT is converted into angiotensin I by renin and subsequently into angiotensin II, a vasoconstrictor, by angiotensin-converting enzymes (Bis JC et al. 2003).

Therefore, we investigated whether that the AGT gene polymorphism may be a risk factor of ischemic stroke in hypertensive subjects.

MATERIALS AND METHODS

Collection of materials:

In presence study, 50 still borne, 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. Then 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 half 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.

8. The slides were stained by eosin for 3-5 min, surplus stain was washed off in water.

9. Dipping into ascending grades of alcohol did the dehydration of slides and clearing was done by two changes of xylene for about 1 min each.

10. The slides were mounted with DPX and cover slips were applied. The slides were kept at room temperature for some hours to allow, firm adhesion of the cover slip to the section.

11. The slides were observed under light microscope for microscopic differentiation and the photographs were taken

12. Few slides, were stained by masson's trichrome technique.

Procedure for measurement of diameters of thyroid follicles:

The stage micrometer scale indicates 1 div=0.01mm = 10 µm.

The micrometer eye piece has only equidistant lines. The line of micrometer scale coinciding with line of micrometer eyepiece is observed and the value of equidistant line is decided. On calculation, 1 division of micrometer eye piece was 4 µm. By using this scale diameters of follicles were calculated at 10x by 40x.

Thus the following parameters were noted:

1) CRL of the Fetuses.

2) Weight of the Fetuses in gms.

3) Weight of the thyroid gland in gms.

4) Microscopic structure of HE stained slides of the thyroid gland.

5) Size of the thyroid follicles was measured.

RESULTS AND DISCUSSION

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.

Table 1: Showing comparisons of body weight in (gms) of present study with the findings of other workers.

Gestational age in weeks	Arey 1954	Gruenwald 1960	Hamilton 1962	Schulz 1962	Potter and Craig 1976	Parulakar 1995	Present study
12	-	-	-	-	-	-	108.3
14	-	-	-	-	38	110	140
16	105	-	73	-	73	200	217
18	-	-	-	-	161	320	266.6
20	310	-	200	312	227	460	266.6
22	-	-	-	-	348	630	528.3
24	640	638	530	7298	361	820	695
26	-	845	-	-	394	1000	925
28	1080	1020	845	1145	-	1300	1216.7
30	-	1230	-	-	1431	1700	1475
32	1670	1488	1940	1778	1900	2100	1780
34	-	1858	-	-	2348	2500	2175
36	2400	2165	3025	2420	2555	2900	2362.5
38	-	2678	-	-	2879	3400	3025

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

Gestational age in weeks	Moore	Hamilton	Potter craig 1976	Schulz 1962	Present study
12	87	57-84	-	-	102.7
14	120	-	-	-	111.5
16	140	61-100	-	-	120.5
18	160	-	-	-	151.7
20	190	101-200	-	227	165
22	210	-	-	-	183
24	230	151-200	209	306	200.4
26	250	-	234	-	221
28	270	201-260	254	-	274
30	280	-	271	-	274
32	300	261-320	284	406	296.7
34	-	-	298	-	298.5
36	340	321-390	324	450	320
38	360	391-450	334	-	327.5

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

Gestational age in weeks	Weight of thyroid (in gms)	
	E. L. Potter	Present study
12	-	0.0717
14	-	0.101
16	-	0.13
18	-	0.166
20	-	0.290
22	-	0.333
24	0.8	0.440
26	0.8	0.475
28	0.8	0.517
30	0.8	0.575
32	0.9	0.716
34	1.1	1.050
36	1.1	1.1
38	1.3	1.25

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 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 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 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.

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.

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 38th 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 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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