CLINICAL STUDY

Biochemical severity of thyroid ectopia in congenital hypothyroidism demonstrates sexual dimorphism

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Abstract

Background: A recent study suggested that sexual dimorphism affects initial thyroid function in congenital hypothyroidism (CH) but differs according to aetiology of CH.

Aims: To determine if sexual dimorphism was associated with biochemical severity of CH and its aetiology in our large British population.

Methods: We examined retrospectively the initial thyroid function tests of 140 infants diagnosed with CH from screening. All infants underwent Tc-pertechnetate radionuclide scans at diagnosis to establish the aetiology of CH prior to commencement of treatment. Patients were classified into athyreosis, ectopia and presumed dyshormonogenesis on the basis of thyroid scans. A comparison of males and females were made within the three aetiological groups for gestational age, birth weight, initial dose of levothyroxine (LT4), screening TSH, confirmatory plasma thyroxine (T4), confirmatory plasma TSH and age of TSH suppression.

Results: There was no significant difference between sexes for gestation, birth weight and initial treatment dose in all aetiological subgroups. In thyroid ectopia, screening TSH and confirmatory plasma TSH were significantly higher in females compared with males ($P < 0.01$), while confirmatory plasma T4 were significantly lower in females ($P < 0.05$). No difference was detected between males and females in athyreosis and dyshormonogenesis subgroups for screening TSH, confirmatory plasma TSH and total T4.

Conclusion: Sexual dimorphism influenced the biochemical severity of thyroid ectopia in congenital hypothyroidism in our British population. However, this effect was not apparent in patients with athyreosis or dyshormonogenesis. Further advances in the molecular genetics of CH are essential to evaluate this phenomenon further.

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Introduction

The overall prevalence of congenital hypothyroidism (CH) is approximately 1 in 3000–4000 births (1). Permanent primary CH are caused by (i) incomplete migration of the thyroid gland, resulting in ectopia; (ii) abnormal differentiation of the gland, resulting in athyreosis or (iii) hormone biosynthetic defects, leading to goitre. The first two entities grouped under the term thyroid dysgenesis are often sporadic, while dyshormonogenesis is often due to autosomal recessive patterns of inheritance (2). There is increasing evidence for the role of sexual dimorphism in CH. Girls are more likely to be affected (3–5). There is also growing evidence that more girls may be affected than boys in thyroid ectopia whilst the sex ratio may be similar in those with absent gland (athyreosis) (6, 7). Boys with CH were reported in one study to have a higher incidence of absent knee epiphyses at diagnosis compared with girls and this sexual dimorphism was demonstrated in all gestational ages (8). A recent study by Eugene *et al.* (3) in a Canadian population of 148 infants with CH found that the severity of CH was worse in girls with thyroid ectopia at

diagnosis, but boys with athyreosis showed marginally poorer biochemical severity compared with girls. No difference in biochemical severity was found for infants with normal thyroid gland (dyshormonogenesis). They hypothesise that sex differences during the foetal period may modulate thyroid gland development and present evidence indicate that sexual dimorphism should be considered in the study of molecular mechanism of thyroid dysgenesis (3). The findings of this group have not been confirmed in any large groups of infants with CH and further studies are needed to evaluate this phenomenon. The aim of the present study was to determine whether sexual dimorphism was demonstrated in the initial biochemical severity of CH dependent on aetiology in a separate large population of British infants.

Methods

We performed a retrospective study evaluating initial thyroid function of 140 infants born between 1982 and 1999 diagnosed with CH at Alder Hey Children's

Hospital, Liverpool diagnosed from a screening programme. The national newborn screening programme for CH was introduced in 1981 to ensure that all infants with CH are identified and effective treatment is started early to prevent lifelong disability. The screening programme covers the regions of Merseyside and Mid-Cheshire in the north-west of England with an estimated 30 000 deliveries per year. Capillary blood samples (blood spot) using heel pricks were collected on filter paper after day 5 of life by midwives in the community in line with nation wide United Kingdom practice. Screening thyroid-stimulating hormone (TSH) (blood spot) of > 20 mU/l was taken as a positive CH screen. Referral was made for formal clinical assessment by the department of endocrinology when a screening test was positive. Investigations to confirm a diagnosis of CH include determination of plasma TSH and total thyroxine (T4) with establishment of aetiology using Tc-pertechnetate radionuclide scans prior to treatment. Infants whose scans showed no radionuclide uptake were diagnosed as athyreosis. Ectopia was diagnosed if scans showed a gland in an abnormal position between the base of the tongue and the normal position of the thyroid gland in the neck. Dyshormonogenesis was diagnosed in infants where uptake of radioisotope was demonstrated in a normally placed thyroid gland. All radionuclide scans were reported by one out of four consultant paediatric radiologists. Treatment was started with 25 mcg/day levothyroxine (LT4) in all patients at diagnosis and adjusted according to biochemical assessments of thyroid function. Follow-up was carried out at 4–6 weeks and at regular intervals until 36 months of age. All children in this study continued to require treatment with LT4 beyond the third year of life. Fifteen (11%) patients with transient hypothyroidism diagnosed on re-testing at 2–3 years of age were excluded from the analysis. Plasma total T4 and TSH were measured using the Delfia two-site fluoroimmuno-metric assay (Delfia T4 and Delfia hTSH Ultra, Wallac Oy, Turku, Finland). The euthyroid reference range based on the Delfia assay for plasma TSH was 0.3–3.8 mU/l. The interassay coefficient of variation for plasma TSH was 2.2% at 17.6 mU/l and 4.8% at 0.045 mU/l. The intraassay coefficient of variation for plasma TSH was 1.3% at 17.6 mU/l and 4.6% at 0.045 mU/l. Adequate TSH suppression

was defined as plasma TSH concentration < 6 mU/l. The normal reference range from our laboratory for total T4 is 138–282 nmol/l from 2 to 10 days and 92–187 nmol/l from 11 to 45 days. The interassay coefficient of variation for T4 was 7.0% at 47 nmol/l. The intraassay coefficient of variation for T4 was 6.3% at 30 nmol/l. SPSS statistical package was used for data analysis. Mann–Whitney *U*-test was used to compare screening TSH, confirmatory plasma TSH, total T4 levels and age of TSH suppression between the sexes for each aetiological group. Data were expressed as median (range).

Results

The distribution of aetiology based on radionuclide scans were as follows: athyreosis (*n*=39), ectopia (*n*=78) and dyshormonogenesis (*n*=23). There was no significant difference between sexes for gestation, birth weight and initial treatment dose (Table 1). No difference was detected between males and females in athyreosis and dyshormonogenesis in terms of screening blood spot TSH, confirmatory plasma TSH and total T4. In contrast, for thyroid ectopia, median screening blood spot TSH was significantly higher in females compared with males (153 vs 101 mU/l, *P*=0.006) and plasma confirmatory TSH levels were also significantly higher in females compared with males (300 vs 118 mU/l, *P*=0.008). Plasma confirmatory total T4 was significantly lower in females compared with males (41 vs 51 nmol/l, *P*=0.02; Table 2). All infants were diagnosed and commenced on LT4 within the first 3 weeks of life. A subgroup analyses combining athyreosis and ectopia found similar results with screening TSH higher in females (*P*=0.01), and confirmatory TSH higher in females (*P*=0.01). Confirmatory T4 was lower in females although this did not reach statistical significance (*P*=0.06).

Discussion

Our distribution of the different aetiologies was also comparable with previous studies (9, 10). Thyroid ectopia has been the most common aetiology and dyshormonogenesis the least common aetiology in

Table 1 Clinical characteristics of patients according to sex and aetiology. Data are presented as median (range).

	Athyreosis		Ectopia		Dyshormonogenesis	
	Males <i>n</i> =15	Females <i>n</i> =24	Males <i>n</i> =24	Females <i>n</i> =54	Males <i>n</i> =11	Females <i>n</i> =12
Gestation (weeks)	40 (39–42)	42 (36–42)	40 (37–43)	41 (37–43)	40 (38–43)	40 (38–42)
Birth weight (kg)	3.8 (3.0–4.2)	3.7 (2.4–5.0)	3.5 (2.4–4.3)	3.6 (2.4–4.8)	3.6 (2.8–4.4)	3.5 (2.8–5.4)
Initial LT4 dose (mcg/kg per day)	6.6 (5.9–8.3)	6.7 (5.0–10.4)	6.8 (5.8–10.4)	6.7 (5.2–10.4)	6.2 (5.6–8.9)	7.5 (4.6–8.9)

LT4, levothyroxine.

Table 2 Thyroid function according to sex and aetiology. Data are presented as median (range).

	Athyreosis		Ectopia		Dyshormonogenesis	
	Males <i>n</i> =15	Females <i>n</i> =24	Males <i>n</i> =24	Females <i>n</i> =54	Males <i>n</i> =11	Females <i>n</i> =12
Screening TSH (mU/l)	170 (8–265)	165 (12–265)	101* (8–260)	153 (26–316)	57 (15–230)	105 (27–244)
Confirmatory TSH (mU/l)	277 (8–680)	329 (12–650)	118* (11–820)	300 (17–760)	47 (12–400)	80 (35–420)
Confirmatory T4 (nmol/l)	17 (6–122)	15 (5–124)	51 [†] (25–159)	41 (11–123)	73 (8–139)	59 (9–80)
Age of TSH normalisation (months)	12.0 (1.8–48.0)	12.0 (6.0–48.0)	12.0 (3.0–86.4)	12.0 (0.8–48.0)	4.0 (1.3–18.0)	3.0 (0.6–24.0)

TSH, thyroid-stimulating hormone; T4, thyroxine. * $P<0.01$ (males versus females); [†] $P<0.05$ (males versus females).

recent studies (7, 9, 10). Our results regarding sex ratios show a female predominance in both athyreosis and ectopia groups, in contrast to Devos *et al.* (8) who found a significant female predominance for ectopia, but not for athyreosis. We have demonstrated in a separate population of infants with CH identified from neonatal screening that sexual dimorphism in the initial biochemical severity of CH was present, but in contrast to the previous study by Eugene *et al.* (3), we found that this was only evident in infants with thyroid ectopia. Girls with thyroid ectopia had higher blood spot screening TSH and plasma TSH at diagnosis with lower plasma T4 at diagnosis. No significant difference in initial biochemical severity between sexes was found for infants with athyreosis or dyshormonogenesis. However, the study by Eugene *et al.* (3) showed that their median TSH was only marginally higher in boys compared with girls in the athyreosis group. Their difference did not reach statistical significance ($P=0.053$). This discrepancy may be due to differences in populations studied and different screening evaluations used. We have previously reported that aetiology of CH was an independent factor affecting suppression of TSH and there were distinct hormonal profiles in response to treatment according to aetiology of CH (11). Our present study shows that responsiveness to treatment with LT4 showed no difference between sexes in the different aetiological groups, as judged by age at suppression of plasma TSH. Studies have also shown that the impact of CH on long-term intellectual development did not differ between boys and girls (10, 12). This suggests that sexual dimorphism as a modulating factor may affect biochemical severity of hypothyroidism by mechanisms occurring in early development, differentiation and migration of the thyroid gland *in utero* and that this does not affect subsequent responsiveness to treatment with LT4. Overall, the starting dose of LT4 in our series of patients used are much lower than present standards and we have now altered our policy of starting infant with no uptake on radionuclide scans on 50 mcg/day LT4 and infants with radionuclide scans suggestive of ectopia and dyshormonogenesis on 37.5 mcg/day LT4. In addition, thyroid function is reassessed in 1–2 weeks

after starting treatment and the dose of LT4 adjusted as appropriate. We have previously reported that there were no significant differences found between males and females in length s.d. and occipito-frontal circumference s.d. within aetiologies from birth to 3 years of age (13). Children with CH identified from neonatal screening treated within the first few weeks of life showed normal growth (14, 15). We conclude that despite the difference in biochemical severity demonstrated between sexes in thyroid ectopia, short periods of postnatal hypothyroidism in these infants identified from screening are probably insufficient to produce a measurable difference in growth between the sexes. In recent years, much progress has been made in the clarification and identification of thyroid defects at a molecular level. Recent advances in molecular genetics have also led to characterisation of numerous genes essential for the normal development of the thyroid gland (16–19). Thyroid disorders may be a polygenic disease or have a multifactorial basis. Permanent causes of CH account for around 90% of cases and originate most frequently from abnormalities of development in thyroid embryogenesis (16). Various forms of thyroid dyshormonogenesis account for the second largest group of permanent cases, with organification defects arising from mutations in the thyroid peroxidase gene which represent the most common form (20, 21). Studies have also found that the TSH receptor gene mutations can present as athyreosis but not all apparently athyreotic patients have this mutation and other molecular mechanism must also be involved. On the other hand, TSH receptor gene mutation is not involved in thyroid migration and is therefore not a candidate for thyroid ectopy. Another cause of CH is a mutation of one of the genes encoding for the thyroid transcription factors: TTF-1, TTF-2 or paired box gene 8 (PAX-8). These have been described as candidate genes for thyroid ectopy, and they play a key role in controlling thyroid gland morphogenesis, differentiation and normal development and migration of the thyroid gland in the foetus (16–19).

Recent advancements in genome sequencing and molecular technology have also provided further insights into the study of genes that are differentially expressed in

each sex in a variety of tissues (20). Males and females appear to respond to disease differently. For example, kidney diseases progress faster in men than in women (21). In systemic lupus erythematosus, a primarily female autoimmune disease, oestrogen has a major role in the progression and development of the disease (22). Males and females appear to have different propensities for cognitive motor skills, suggesting that there might be further variation in brain structure (23). Many questions remain regarding the molecular contributions to the dimorphic physiologies of males and females.

It has been suggested that only 2% of patients with thyroid dysgenesis incorporating the spectrum of ectopia and athyreosis have a familial basis (16, 24). The female preponderance was observed in sporadic forms of thyroid dysgenesis with almost thrice the number of girls affected (3, 6). However, the M:F ratio is similar in familial forms of thyroid dysgenesis (16). It is possible that the effect of sexual dimorphism on initial thyroid function may be different between the familial and the sporadic forms of thyroid dysgenesis. Due to the retrospective nature of our study, we were unable to accurately determine the proportion of children in our study who had familial forms of thyroid dysgenesis. Based on the reported frequency of approximately 2% of familial thyroid dysgenesis (16, 24), we would only expect 2 patients in our series of 117 patients with ectopia and athyreosis to have a familial basis, thus making any form of statistical analysis of the familial cases impossible even if we were able to identify them.

The determination of aetiology of CH in our study is based on results of radionuclide technetium scan at diagnosis prior to treatment similar to the method used in the previous study by Eugene *et al.* (3). Future studies may need to include patients where stricter criteria are used for the establishment of aetiology including assessment with thyroid ultrasound, plasma thyroglobulin levels in the infant and the maternal blocking antibodies are used. It is known that infants with no uptake on radionuclide scan may have presence of hypoplastic thyroid gland on ultrasound and normal levels of thyroglobulin (25, 26). These patients are presently classified as apparent athyreosis. Only 42% of infants with no uptake on radionuclide scan have undetectable thyroglobulin levels at diagnosis, indicating 'true athyreosis' (25). A recent study suggests that in 15% of infants (6 out of 40) with no uptake on radionuclide scan, thyroid ultrasound detected the presence of thyroid tissue. Four out of the six infants had permanent hypothyroidism: three hypoplastic gland, one hemiagenesis; one transient hypothyroidism due to maternal blocking antibodies and one status still unclear (27). Whether thyroid ectopia, true athyreosis and apparent athyreosis are distinctly separate entities or a spectrum of similar developmental thyroid abnormality remains uncertain. In our subgroup analyses combining athyreosis and ectopia, we found similar results with worse initial biochemical severity of

CH in girls. Future studies on the influence of sexual dimorphism on the severity of hypothyroidism in the aetiology of CH may need to address these factors to explain the difference in results for our patients with athyreosis compared with the previous study by Eugene *et al.* (3). This is important in answering the question of whether the biochemical severity of hypothyroidism is only sexually dimorphic in infants with ectopia alone or throughout the spectrum of thyroid dysgenesis, incorporating, true athyreosis, apparent athyreosis and ectopia. This is the only study confirming the evidence reported by Eugene *et al.* (3) that sexual dimorphism influences biochemical severity of CH at diagnosis but our results differed from their data because this was only apparent in patients with thyroid ectopia. Both our study and the study by Eugene *et al.* (3) were of similar sample size. Larger studies are needed to assess sexual dimorphism in familial and sporadic forms of thyroid dysgenesis and the impact of initial biochemical severity. Implications of present findings may be that girls may require higher doses of initial replacement therapy. In conclusion, our data demonstrates in a separate population of British infants with CH that sexual dimorphism influenced the initial biochemical severity of thyroid ectopia in congenital hypothyroidism but this was not demonstrated in children with athyreosis and dysmorphogenesis. Our results suggest that sex determining factors may modulate the genetic and molecular mechanism of thyroid gland migration in thyroid ectopia leading to differing plasma TSH and T4 at diagnosis depending on the sex of the infant. Whether this difference is due to a reduction of normal functional thyroid tissue in girls with ectopia is still unknown. Future developmental and molecular genetics studies on the aetiology of thyroid ectopia in CH need to take into account the effect of sexual dimorphism.

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