

# Alphafetoprotein in screening for congenital hypothyroidism

R.M. Shawky,<sup>1</sup> S. Abd El-Fattah,<sup>1</sup> M.E. El-din Azzam,<sup>2</sup> M.M. Rafik<sup>3</sup> and A. Osman<sup>1</sup>

## استعمال البروتين الجنيني ألفا في تحريي قصور الدرقية الخلقي

رباح محمد شوقي وشيرين عبد الفتاح ومحمد عز الدين عزام ومنى محمد رفيق وأمان عثمان

**خلاصة:** أجريت هذه الدراسة على خمسمئة من الولدان الحديثي الولادة الذين أتموا مدة الحمل وعلى خمسة وعشرين طفلاً أكبر سناً من المصابين بقصور الدرقية الخلقي المشخص حديثاً أو من قبل. ووجد أن البروتين الجنيني ألفا كان مرتفعاً لدى اثنين من الولدان. ووجد في أحدهما ارتفاع مستمر في البروتين الجنيني ألفا وفي الهرمون المنشط للدرقية، مع انخفاض مستوى الثيروكسين (قصور الدرقية الخلقي). أما الوليد الثاني فقد عاد فيه إلى المستويات الطبيعية كل من البروتين الجنيني ألفا والهرمون المنشط للدرقية والثيروكسين (قصور درقي عابر). وكان المستوى الوسطي للبروتين الجنيني ألفا في المرضى الجدد بقصور الدرقية الخلقي أعلى، بدرجة يعتد بها إحصائياً من أولئك الذين شُخصوا من قبل، كما كان أعلى في المرضى عنه في المجموعة الشاهدة. وقد وجدت علاقات يُعتد بها بين البروتين الجنيني ألفا والثيروكسين، وبينه وبين الهرمون المنشط للدرقية، وبينه وبين العمر. إن البروتين الجنيني ألفا مُشعرٌ (مؤشرٌ) حساس لحالة الغدة الدرقية، ويمكن أن يُستعمل كاختبار لتحريي قصور الدرقية منذ اليوم الأول للحياة، وفي متابعة حالات المصابين بقصور الدرقية الخلقي.

**ABSTRACT** This study was conducted on 500 full-term neonates and 25 older patients with congenital hypothyroidism (CH), newly or previously diagnosed. Alphafetoprotein (AFP) was elevated in two neonates. In one, persistent elevation of AFP and thyroid stimulating hormone (TSH) with low thyroxine ( $T_4$ ) were found (congenital hypothyroidism). In the other, AFP, TSH and  $T_4$  levels normalized (transient hypothyroidism). The mean AFP level in new CH patients was significantly higher than in previously diagnosed patients, and was higher in CH patients than in controls. Significant relationships were found between AFP and  $T_4$ , AFP and TSH, and AFP and age. AFP is a sensitive indicator of thyroid status and can be used as a screening test for hypothyroidism from the first day of life and in follow-up of CH patients.

## L'alphafetoprotéine dans le dépistage de l'hyperthyroïdie congénitale

**RESUME** Cette étude a été réalisée sur 500 nouveau-nés à terme et 25 patients plus âgés atteints d'hyperthyroïdie congénitale, nouvellement ou précédemment diagnostiquée. L'alphafetoprotéine (AFP) était élevée chez deux nouveau-nés. Chez un de ces nouveau-nés, on a trouvé une élévation de l'AFP et de l'hormone de libération de la thyrostimuline (TSH) avec une faible thyroxine ( $T_4$ ) (hyperthyroïdie congénitale). Chez l'autre, les niveaux d'AFP, de TSH et de  $T_4$  s'étaient normalisés (hyperthyroïdie transitoire). Le niveau d'AFP moyen chez les nouveaux patients atteints d'hyperthyroïdie congénitale était significativement plus élevé que chez les patients pour lesquels le diagnostic avait été posé précédemment, et il était plus élevé chez les patients atteints d'hyperthyroïdie congénitale que chez les témoins. Une relation significative a été trouvée entre l'AFP et la  $T_4$ , l'AFP et la TSH et l'âge. L'AFP est un indicateur sensible du statut thyroïdien et peut être utilisée comme test de dépistage de l'hyperthyroïdie dès le premier jour de la vie et durant le suivi des patients atteints d'hyperthyroïdie congénitale.

<sup>1</sup>Department of Paediatrics; <sup>2</sup>Department of Gynaecology and Obstetrics; <sup>3</sup>Department of Clinical Pathology, University of Ain Shams, Cairo, Egypt.

Received: 19/06/00, accepted: 18/09/00

## Introduction

Alphafetoprotein (AFP) is a single-chain alpha globulin produced in the yolk sac and liver of developing fetuses. When the yolk sac degenerates at 12 weeks post-conception, AFP is produced exclusively by the fetal liver at an increased rate until 13–14 weeks, then declines gradually towards term. It is excreted in fetal urine and can be easily detected in the amniotic fluid after 10 weeks' gestation. AFP diffuses across fetal membranes (it may also be transported via the placental circulation) and is usually found in maternal serum after 12 weeks [1].

A relationship has been found between neonatal serum AFP and congenital hypothyroidism (CH). Elevated neonatal serum AFP levels were present in association with elevated thyroid stimulating hormone (TSH) levels and/or lowered thyroxine ( $T_4$ ) levels. These situations may contribute to the persistence of elevated AFP in the neonatal period as a result of increased synthesis or delayed repression of AFP synthesis in infants with CH [2]. Ben Neriah et al. suggested an association between CH and high maternal serum AFP [3]. However, normal maternal serum AFP has been found in pregnancies affected by CH [4,5].

We aimed to determine the accuracy of neonatal serum AFP levels as a screening test for CH. The AFP level was also determined in selected older patients with hypothyroidism. Correlations between the serum level of AFP and levels of TSH and  $T_4$  were performed to determine the reliability of AFP as a screening test for CH.

## Patients and methods

This study was conducted in two groups of children. Group 1 consisted of 500 clinically apparently normal neonates from the

inpatient ward of the Department of Obstetrics and Gynaecology and the Paediatric outpatient clinic at the University of Ain Shams. There were 250 males and 250 females with an age range of 1–25 days and a mean age  $\pm$  standard deviation of  $4.05 \pm 1.74$  days. Exclusion criteria included prematurity, intrauterine growth retardation, generalized oedema, jaundice, neural tube defects, hepatomegaly, exomphalos and infants born of hypertensive or diabetic mothers.

Group 2 included 25 CH patients (16 males and 9 females) with an age range from 6 months to 21 years and a mean age of  $7.5 \pm 6.5$  years. This group was further subdivided into two groups:

- New CH patients: 15 patients (10 males and 5 females) who were referred to the Children's Hospital with clinical signs and symptoms suggestive of CH.
- Old CH patients: 10 patients (6 males and 4 females) who had stopped receiving or were receiving irregular or inadequate doses of replacement therapy (L-thyroxine).

Ten apparently normal children matched for age and sex were included in the study as controls.

The patient's age, sex, address and gestational age (for neonates) were recorded. Prenatal, natal and postnatal history was obtained with special emphasis on maternal diseases (thyrotoxicosis or hyperthyroidism), maternal intake of drugs (especially antithyroid drugs such as neomercazole) during pregnancy, and family history of thyrotoxicosis, hypothyroidism or goitre. The pedigree was constructed.

A thorough clinical examination was conducted with special emphasis on signs of hypothyroidism, such as large protruding tongue, dull apathetic appearance (these signs are rarely encountered in neonates with CH), goitre, skull circum-

ference, anterior and posterior fontanelles, neural tube defects, hepatomegaly, jaundice and generalized oedema.

A skeletal survey was carried out for all CH patients (new and old) to detect radiological features of hypothyroidism and any skeletal anomalies. Determination of bone age by X-ray of the left hand and wrist was also performed.

M-Mode, two-dimensional and Doppler echocardiography and auditory brain stem evoked responses (ABR) were performed on all CH patients (new and old). Abdominal ultrasound was performed for all CH patients with associated congenital anomalies.

AFP [6,7], TSH [6] and  $T_4$  [6] were determined. Samples taken before the third day of life were used for AFP assay only, to avoid the effect of TSH surge. Patients with high AFP on the first day of life were recalled for assessment of  $T_4$  and TSH after the third day of life [8]. Alanine aminotransferase (ALT), bilirubin (total and direct), creatinine and creatine kinase (Beckman Instruments Incorporated, California, United States of America) were also determined.

Statistical analysis was performed using Student *t*-test, chi-squared test, Mann-Whitney and Fisher exact *P* value.

## Results

### Group 1

The mean AFP of neonates was  $141.9 \pm 89.9$  ng/mL. There was no statistically significant difference between the mean AFP of males ( $142.3 \pm 91.6$  ng/mL) and females ( $141.6 \pm 88.3$  ng/mL) ( $P > 0.05$ ).

The normal AFP during the first 2 months of life does not exceed 400 ng/mL [9,10]. The serum AFP levels of two neonates (one male and one female aged 1 and 4 days respectively) exceeded this value, and further laboratory tests were therefore performed (Table 1). In the male patient, the elevation of AFP and TSH with low  $T_4$  levels persisted after the third day of life (associated with normal ALT, creatinine and bilirubin) (Table 1). The pedigree of this patient showed no consanguinity. Clinically the patient was hypoactive and constipated.

Table 1 Laboratory investigations of two neonates with elevated alphafetoprotein (AFP)

| Parameter        | Normal value   | Male | Female |
|------------------|--|------|--------|
| AFP              | 400 ng/mL  | 469  | 431    |
| $T_4$            | < 6 µg/dL  | 1.2  | 1.2    |
| TSH              | < 25 mU/L (at 3 days old)<br>< 20 mU/L from 4 days onwards [2] | 46   | 24     |
| ALT              | 6–50 U/L   | 28   | 14     |
| Creatinine       | 0.3–1 mg/dL  | 0.3  | 0.6    |
| Total bilirubin  | 0.2–1 mg/dL  | 0.4  | 0.2    |
| Direct bilirubin | 0–0.2 mg/dL  | 0.1  | 0      |

$T_4$  = thyroxine.

TSH = thyroid stimulating hormone.

ALT = alanine aminotransferase.

In the female patient repetition of laboratory investigations at the age of 7 days gave normal values of AFP, TSH,  $T_4$ , ALT, creatinine and bilirubin and the patient was considered to be a case of transient hypothyroidism. Thus, out of 500 neonates screened, one had permanent primary CH.

## Group 2

The clinical presentations of this group are shown in Figure 1. The commonest was constipation (52%) and the least common was delayed closure of fontanelles (4%). Most patients presented with a combination of signs and symptoms.

Echocardiography revealed patent ductus arteriosus in a new female patient aged 3 years and predominant ventricular septal hypertrophy in a new female patient aged 1 year. ABR revealed hearing loss in two patients (bilateral moderate severe hearing loss in a new male patient aged 7 months, and left severe hearing loss with right moderately severe hearing loss in a new female

patient aged 3 years). The skeletal survey detected a mean retardation in bone age of  $3.09 \pm 2.61$  years, wormian bones in the skull in 3 patients, thickened skull vault in 2 patients, widened sella turcica in 2 patients, delayed dentition in 5 patients, open fontanelles in 2 patients, beaked vertebrae in 18 patients, spina bifida in 14 patients, and epiphyseal affection in 9 patients. Abdominal sonography did not show any congenital anomalies in the CH patients studied.

The laboratory results of CH patients are shown in Table 2. Creatine kinase levels were high in 3 male patients with Kocher-Debré-Semelaigne syndrome who presented with pseudohypertrophy, especially in the calf muscles, associated with muscle weakness. Two patients were aged 18 years, and one aged 20 years. Their creatine kinase levels were 155, 182 and 176 U/L (normal level = 5–130 U/L).

Figures 2, 3 and 4 show the correlation between AFP and age in years, levels of  $T_4$  and levels of TSH in patients with CH.

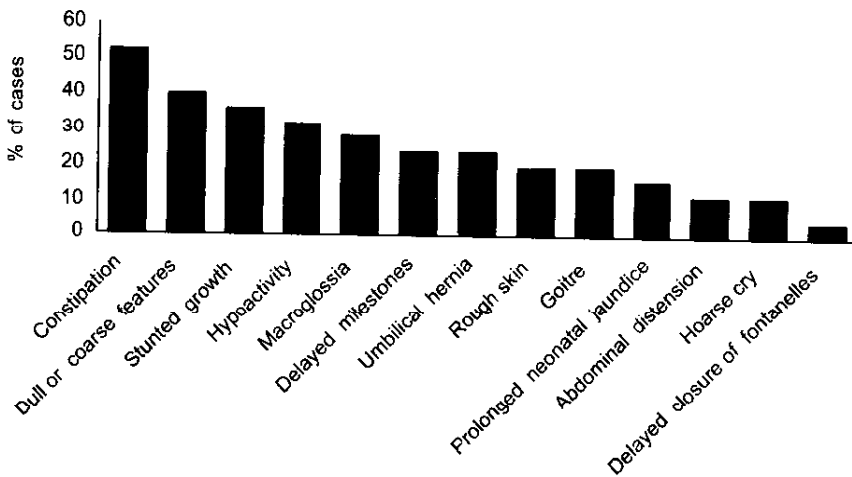


Figure 1 Clinical presentation of patients with congenital hypothyroidism.

Table 2 Laboratory investigations of patients with congenital hypothyroidism

| Measurement            | Range    | Mean               | s     | Normal control  |
|------------------------|----------|--------------------|-------|---|
| Age (years)            | 0.5–21   | 7.5                | 6.5   |   |
| AFP (ng/mL)            | 166–695  | 366.2 <sup>a</sup> | 137.4 | 25–30   |
| T <sub>4</sub> (µg/dL) | 0.1–11.5 | 4.35               | 3.78  | 4.3–12.5  |
| TSH (mU/L)             | 10.9–255 | 68.6               | 46.6  | 0.2–6.3   |
| ALT (U/L)              | 10–42    | 24.52              | 4.35  | 5–45  |
| Creatinine (mg/dL)     | 0.2–0.8  | 0.47               | 4.35  | 0.2–0.4 (infants)<br>0.3–0.7 (children)<br>0.6–1.2 (adult male)<br>0.5–1.1 (adult female) |
| Bilirubin (mg/dL)      |          |                    |       |   |
| Total                  | 0.2–1    | 0.54               | 0.22  | 0.2–1   |
| Direct                 | 0–0.2    | 0.096              | 0.084 | 0–0.2   |

<sup>a</sup>The mean AFP level for male patients with congenital hypothyroidism ( $366.8 \pm 143.4$  ng/mL) was statistically nonsignificantly different from that of female patients with congenital hypothyroidism ( $366.8 \pm 134.4$  ng/mL) ( $P > 0.05$ ).

AFP = alphafetoprotein.

TSH = thyroid stimulating hormone.

s = standard deviation.

T<sub>4</sub> = thyroxine.

ALT = alanine aminotransferase

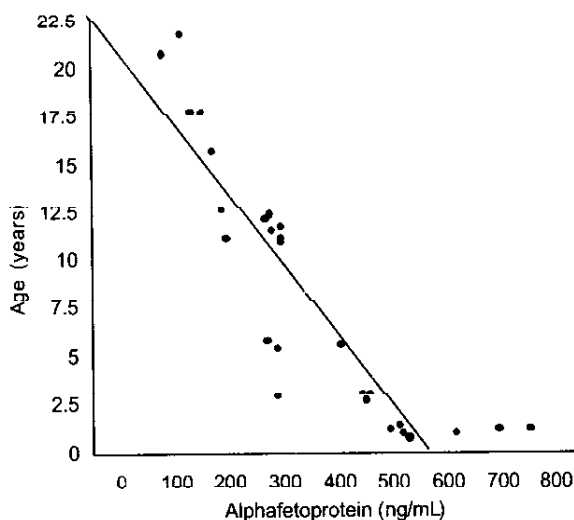
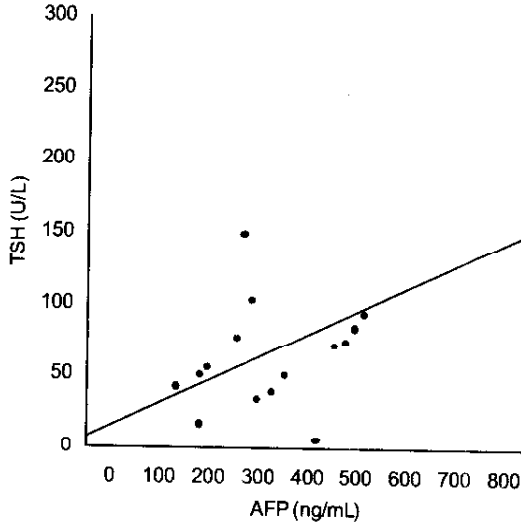
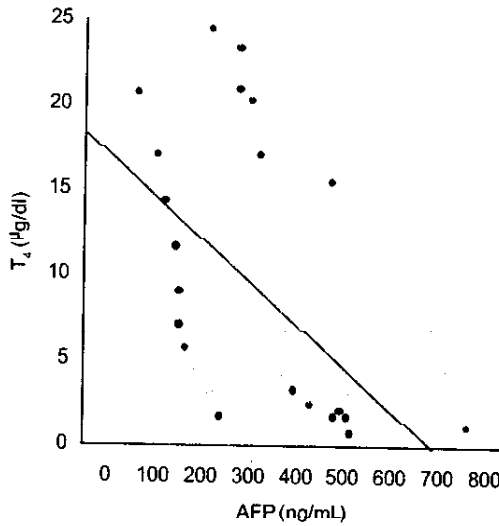


Figure 2 Correlation between alphafetoprotein (AFP) levels and age in patients with congenital hypothyroidism. AFP level was significantly inversely related to age ( $r = -0.83$ ,  $P < 0.001$ ).



**Figure 3** Correlation between alphafetoprotein (AFP) and thyroxine ( $T_4$ ) levels in patients with congenital hypothyroidism. AFP level was significantly inversely related to  $T_4$  level ( $r = -0.654$ ,  $P < 0.001$ ).



**Figure 4** Correlation between alphafetoprotein (AFP) and thyroid stimulating hormone (TSH) levels in patients with congenital hypothyroidism. AFP level was significantly directly related to TSH level ( $r = 0.49$ ,  $P < 0.005$ ).

## Discussion

In our study, AFP levels were determined in 500 apparently clinically normal full-term neonates. Their mean AFP level ( $141.9 \pm 89.9$   $\mu\text{g/mL}$ ) was within the normal reference range [10]. The mean AFP level for full-term male neonates ( $142.3 \pm 91.6$   $\text{ng/mL}$ ) was statistically nonsignificantly different from full-term females ( $141 \pm 88.3$   $\text{ng/mL}$ ) ( $P > 0.05$ ), which is in agreement with Willi and Moshang [11] and Mizejewski et al. [12].

AFP levels higher than the reference value were associated with low  $T_4$  and high TSH in two neonates (a male and a female). Repetition of the investigation revealed a persistent elevation of TSH with low  $T_4$  in the male (primary CH), while both TSH and  $T_4$  normalized in the female (transient hypothyroidism). Transient hypothyroidism is defined as abnormal thyroid function on initial screening which improves on follow-up [13]. Thus, the female neonate was considered to be a case of transient hypothyroidism, due to the use of iodine as a disinfectant; the passing of the effect of iodine led to the correction of the transient impairment of thyroid function, and normalization of TSH,  $T_4$  and AFP levels. Thus, primary CII was confirmed in only one neonate out of the 500 screened.

Immediately after birth, there is a rapid TSH surge (stimulating  $T_4$  production) that peaks at about 30 minutes after labour. Thereafter, there is a dramatic fall in TSH up to about 12 hours, followed by a gradual stabilization at about 48 hours of life [14]. CH screening programmes encounter the problem of early maternal-neonatal postpartum discharge, whether the initial method of screening is by TSH and/or  $T_4$  [1], as a result of the immediate TSH surge leading to false-positives (i.e. falsely high TSH) or false-negatives (falsely high  $T_4$ ). In both

these cases, the AFP level will be normal as it is elevated only in the presence of high TSH and low  $T_4$  [15].

False-positive and false-negative cases are not due to thyroid dysfunction and are accordingly associated with normal AFP levels. Using AFP as the primary method of screening for CH would allow early collection of screening samples. Where samples show high AFP levels, the age at sample collection should be rechecked. If it was taken within the first 48 hours of birth, it cannot be used for TSH and  $T_4$  estimation since it will reflect the TSH surge. If the sample was taken from the third day of life onwards, it can be used for TSH and  $T_4$  estimation since the postnatal hyperthyrotropinaemia will have subsided [16].

The clinical presentations of hypothyroidism are numerous. In our group of CH patients, the following signs and symptoms were found in the percentages indicated, compared to the percentages found by others [13,17,18]: constipation was found in 52% of our patients versus 14%, dull or hypothyroid features in 40% versus 4%, hypoactivity in 32% versus 23%, macroglossia in 28% versus 18%, umbilical hernia in 24% versus 25%, prolonged neonatal jaundice in 16% versus 46% and hoarse cry in 12% versus 7%.

Echocardiography performed on CH patients revealed patent ductus arteriosus in one new patient and predominant ventricular septal hypertrophy in another. A study by Siebner et al. on 943 CH patients found 14 patients with associated cardiac lesions: ventricular septal defects, patent ductus arteriosus, pulmonary stenosis, mitral insufficiency and congenital atrial flutter [19]. Both the conditions found by echocardiography in our two patients reversed after 6 months of L-thyroxine replacement therapy, which is in agreement with other authors [20,21].

Two new CH patients had hearing loss, and one of them was diagnosed as a case of Pendred syndrome, which is responsible for 5% of all cases of congenital deafness [22]. Abnormally low circulating levels of thyroid hormones during fetal life and the first postnatal weeks may impair the development of the cochlea and the organ of Corti leading to sensory neural hypoacusis in CH [23,24].

Pathological changes at the growth plate seen in hypothyroidism include a marked decrease in cartilage cell proliferation, producing the radiological manifestations of retarded skeletal maturation [25]. In cases of CH, skull changes may be evident. Wormian bones were detected in three older patients, which is in line with the findings of Caffey and Silverman [26].

Dentition was delayed in two new and three old patients, again in line with Caffey and Silverman, who stated that dental development in hypothyroidism is delayed, although not to the extent that occurs in skeletal maturation [26].

Open fontanelles were found in two new patients (aged 3 years). Smith and Popich suggested that a large anterior fontanelle or an open posterior fontanelle of unexplained origin could be an initial indicator of hypothyroidism [27]. A thickened skull vault was detected in one new and another old patient, a feature that has been described in cases of hypothyroidism [28]. Widened sella turcica was detected in two old patients. Vogler and Genant explained this by rebound hypertrophy and hyperplasia of the pituitary gland due to inadequate feedback from the hypofunctioning thyroid gland [25]. Beaked vertebrae in the dorsolumbar region were evident in eight new and ten old patients, which has been reported in cases of hypothyroidism [29]. Epiphyseal affection occurred in the form of epiphyseal dysgenesis (multiple foci of os-

sification) in four new and five old patients. Epiphyseal centres in the proximal tibia and distal femur were absent in two patients (normally present at birth), indicating deprivation of thyroid hormone during intrauterine life [13].

AFP levels were significantly higher in the second group (CH patients) ( $366.2 \pm 137.4$  ng/mL) than in controls ( $P = 0.001$ ). The AFP level was also statistically significantly higher in new ( $450.9 \pm 109.4$  ng/mL) than in old CH patients ( $239 \pm 42.2$  ng/mL) ( $P = 0.001$ ).

In our study, AFP was significantly inversely related to age ( $r = -0.83$ ,  $P < 0.001$ ), with younger patients having higher AFP levels, and to  $T_4$  ( $r = -0.54$ ,  $P = 0.001$ ), while it was significantly directly related to TSH levels ( $r = 0.49$ ,  $P < 0.05$ ) in CH patients. These results are in agreement with several authors [15,30].

The physiological mechanism by which serum AFP is elevated in CH has been suggested to be either an increased rate or delayed repression of AFP synthesis by the liver [15]. Other authors have suggested that the half-life of AFP is extended in hypothyroid patients [30]. They postulate that low  $T_4$  levels may be responsible for a retarded catabolism of serum AFP in the liver.

## Conclusion

The level of AFP protein is a sensitive indicator of thyroid status. AFP estimation could therefore serve as a reliable screening test for CH, as it is elevated in cases of low  $T_4$  and high TSH. Samples for AFP assessment could be taken in the first day of life, since AFP is not affected by the TSH surge, which delays the taking of screening samples for CH for at least 48 hours. Additionally, AFP is very helpful in confirming the diagnosis and in the follow-up of therapy in patients with CH.



### References

1. Allen DB et al. Screening programs for congenital hypothyroidism. How can they be improved? *American journal of diseases of children*, 1988, 142:232-6.
2. Allen DB et al. Age-adjusted thyrotropin criteria for neonatal screening for congenital hypothyroidism. *Journal of pediatrics*, 1990, 117:309-12.
3. Ben-Neriah Z et al. Increased maternal serum alpha-fetoprotein in congenital hypothyroidism [Letter]. *Lancet*, 1991, 337:437.
4. Burton BK. Outcome of pregnancy in patients with unexplained elevated or low levels of maternal serum alpha-fetoprotein. *Obstetrics and gynecology*, 1988, 72:709-13.
5. Maternal serum alpha-fetoprotein in congenital hypothyroidism [Letter]. *Lancet*, 1991, 337:922.
6. Chieragatti G et al. The "soluble sandwich" approach for immunoassays: methodological and instrumental implications. *Annales de biologie clinique*, 1990, 48:393-7.
7. Seppala M, Ruoslahti E. Radioimmunoassay of maternal serum alpha fetoprotein during pregnancy and delivery. *American journal of obstetrics and gynecology*, 1972, 112:208-12.
8. LaFranchi SH et al. Screening for congenital hypothyroidism with specimen collection at two time periods: results of the Northwest Region Screening Program. *Pediatrics*, 1985, 76:734-40.
9. Nicholson JF, Pesce MA. Laboratory testing and reference values in infants and children. In: Behrman RE, Kliegman RM, Vaghan VC, eds. *Nelson textbook of pediatrics*, 14th ed. Philadelphia, WB Saunders Company, 1992:1797-826.
10. Wachnin S. The clinical significance of human alphafeto-protein. *Annals of clinical laboratory science*, 1978, 8:84-90.
11. Willi SM, Moshang T. Diagnostic dilemmas. Results of screening tests for congenital hypothyroidism. *Pediatric clinics of North America*, 1991, 38(3):555-66.
12. Mizejewski GJ et al. Measurement of serum alpha-fetoprotein in early infancy: utilization of dried blood specimens. *Pediatric research*, 1983, 17:47-50.
13. Grant DB et al. Congenital hypothyroidism detected by neonatal screening: relationship between biochemical severity and early clinical features. *Archives of disease in children*, 1992, 67:87-90.
14. Fisher DA, Odell WD. Acute release of thyrotropin in the newborn. *Journal of clinical investigation*, 1969, 48:1670-7.
15. Mizejewski GJ, Pass KA. Alpha-fetoprotein and hypothyroidism in infants. *Pediatrics*, 1992, 90(6):1008-9.
16. Delange F et al. Progress report on neonatal screening for congenital hypothyroidism in Europe. In: Burrow GN, Dusault JH, eds. *Neonatal thyroid screening*. New York, Raven Press, 1980: 107-31.
17. Khaleeli AA, Griffith DG, Edwards RH. The clinical presentation of hypothyroid myopathy and its abnormalities in structure and function of skeletal muscle. *Clinical endocrinology*, 1983, 19:365-76.
18. Najjar SS. Muscular hypertrophy in hypothyroid children: the Kocher-Debré-Semelaigne syndrome. A review of 23 cases. *Journal of pediatrics*, 1974, 85: 236-9.

19. Siebner R et al. Congenital anomalies concomitant with persistent primary congenital hypothyroidism. *American journal of medical genetics*, 1992, 44(1): 57-60.
20. Nakagawa T. Delayed closure of ductus arteriosus in premature infants with transient hypothyroidism [Letter]. *Lancet*, 1993, 341:839.
21. Santos AD et al. Echocardiographic characterization of the reversible cardiomyopathy of hypothyroidism. *American journal of medicine*, 1980, 68:675-82.
22. Kabakkaya Y et al. Pendred's syndrome. *Annals of otology, rhinology, and laryngology*, 1993, 102(4 Pt 1):285-8.
23. Deol MS. The role of thyroxine in the differentiation of the organ of Corti. *Acta oto-laryngologica*, 1976, 81:429-35.
24. Uziel A et al. Effects of hypothyroidism on the structural development of the organ of Corti in the rat. *Acta otolaryngologica*, 1981, 92:469-80.
25. Vogler JB, Genant HK. Skeletal metabolic diseases. In: Grainger RG, Allison DJ, eds. *Diagnostic radiology*, 2nd ed. Edinburgh, Churchill Livingstone, 1992:1627-8.
26. Caffey J, Silveiman FN, eds. *Skeletal changes in the endocrinopathies and normal vertebral column. Pediatric X-ray diagnosis*. Chicago, Yearbook Medical Publishers Incorporated, 1970:1050-4, 1101-8.
27. Smith DW, Popich G. Large fontanels in congenital hypothyroidism: a potential clue toward earlier recognition. *Journal of pediatrics*, 1972, 80:753-6.
28. Keats TE, Alford BA. The muscul-skeletal system in children. In: Grainger RG, Allison DJ, eds. *Diagnostic radiology*, 2nd ed. Edinburgh, Churchill Livingstone, 1992:1729.
29. Young. The thyroid gland. In: Sutton D, ed. *A textbook of radiology and imaging*. Edinburgh, Churchill Livingstone, 1992: 229-30.
30. Mengreli C et al. Alpha-fetoprotein in congenital hypothyroidism. *Biology of the neonate*, 1990, 58:326-33.