대한 유전성 대사 질환 : 2019;19(1):1~11

원 저

한국인 선천성 갑상선기능저하증에 대한 신생아선별검사의 14년간의 후향적 연구; 발생빈도와 유효성

덕성여자대학교 약학대학 생의약분석실[1], 서울의과학연구소 특수분석팀[2]

윤혜란[1] · 안선현[2] · 이향자[2]

Evaluation of the Congenital Hypothyroidism for Newborn Screening Program in Korea: A 14-year Retrospective Cohort Study

Hye-Ran Yoon[1], Sunhyun Ahn[2], Hyangja Lee[2]

Pharmaceutical & Biomedical analysis Lab[1], Duksung Women's University, Seoul, Korea
Department of Special Chemistry[2], Seoul Clinical Laboratories (SCL), Yongin, Korea

Purpose: Congenital hypothyroidism (CH) is the most common congenital endocrine disorder. The purpose of the present study was to determine the incidence of CH in South Korea during the period from January 1991 to March 2004.

Methods: Central data from each city branch of SCL (Seoul Clinical Reference Laboratories) in Yongin, South Korea, was gathered and collectively analyzed. Newborn screening (NBS) for CH was based on measuring the levels of neonatal thyroid stimulating hormone (TSH) and free T4 (a cut-off of 20 mIU/L and less than 0.8 ng/dL, respectively).

Results: During the study period, 671,805 live births were screened for CH based on TSH and free T4 ELISA assays. A total of 159 newborns were deemed positive for CH out of 671,805, with a corresponding incidence of 1 in 4,225. When a cut-off of 20 mIU/L was used in TSH assays, the associated sensitivity, specificity, and positive predictive values (PPV) were 100.0%, 99.7%, and 10.8%, respectively. When a cut-off of 0.8 ng/dL in free T4 assays was used, the associated sensitivity, specificity, and PPV were 100.0%, 98.5%, and 3.9%, respectively.

Conclusion: CH incidence in South Korea as evidenced by the results of NBS was compared with its incidence and comparable to the other countries prior to 2004.

Key words: Congenital hypothyroidism, Thyroid gland, Newborn screening, Evaluation, Prevalence

Introduction

Congenital hypothyroidism (CH) results from inadequate thyroid hormone production in newborn infants and is a condition which affects them from birth, resulting in the loss of thyroid function (hypothyroidism), usually due to the incorrect development of the thyroid gland[1-3]. Underactive thyroids can occur in newborns due to anatomic defects in the gland itself (absent or ectopic), an intrinsic hindrance to thyroid metabolism, or iodine deficiency (Fig. 1). An underactive thyroid gland results in the inadequate production of thyroxine/T4 after birth[4], which can affect mental and phy-

책임저자: 윤혜란, 서울특별시 도봉구 삼양로 144길 33
덕성여자대학교 약학대학 312호
Tel: 02)901-8387, Fax: 02)901-8386
E-mail: hyeran11@duksung.ac.kr

sical development resulting in reduced mental function. Mental retardation caused by hypothyroidism can be prevented if the disorder is diagnosed and treated early. The worldwide prevalence of CH is around 1 in 2,000-4,000 births[1-4].

In most industrialized societies, newborn CH screening has been an integral part of public health programs for several decades; however, several unresolved clinical issues still remain. Population-wide CH screening has undergone significant changes since it was first introduced in North America in 1975 and its subsequent implementation across Europe[5-8], Australia[9,10], Japan[11], China [12,13], and other countries[14-18]. Gradually, CH newborn screening in newborns has been implemented worldwide.

CH screening was first introduced in South Korea in 1985. Newborns were screened for 5 diseases including CH by the Mother and Child Health Association in 1991[11]. The incidence of CH is around 1 in every 4,000 births in South Korea[11]. There have been no reports concerning the association between newborn screening methods and CH incidence for at least a decade in Korea.

In this study, the sensitivity, specificity, and positive predictive value of TSH and free T4 assays were evaluated retrospectively and the prevalence of CH in Korea was compared with that of overseas countries.

Materials and Methods

1. Collection of specimens and management

Heel prick dried blood spot specimens (2.5 mm punch size) were taken within 5 days of birth.

Seoul city and 6 major cities (Busan, Incheon, Daegu, Daejeon, Gwangju, and Ulsan cities), and 9 provinces (Kyunggi, Kangwon, Chungbuk, Chungnam, Jeonbuk, Jeonam, Gyeongbuk, Gyeongnam,

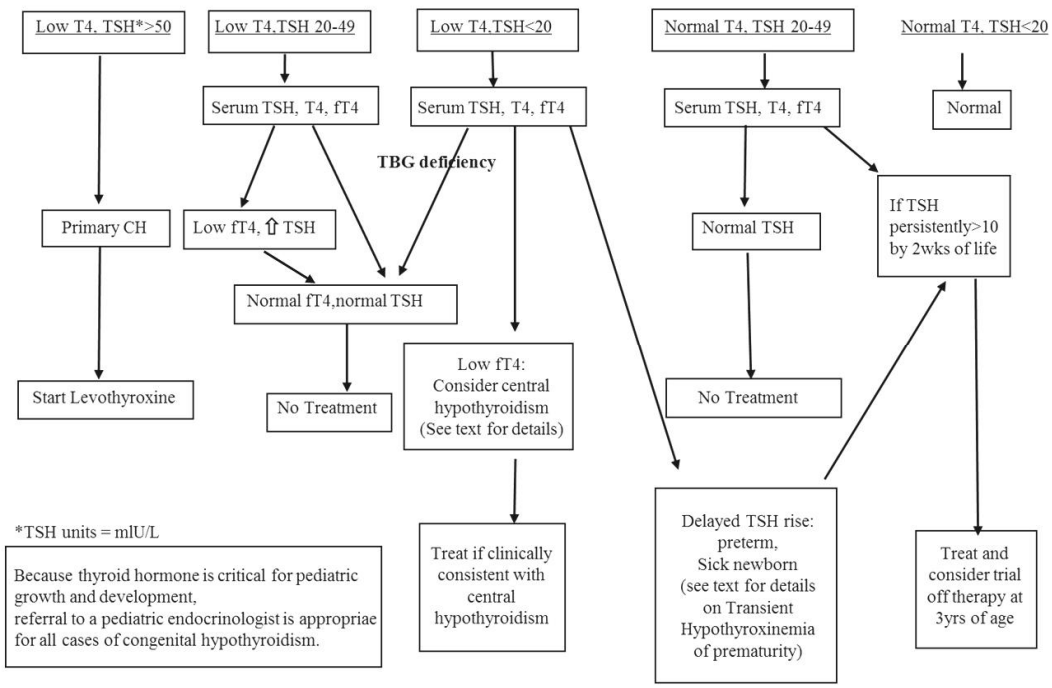


Fig. 1. Algorithm for the diagnosis of congenital hypothyroidism.

and Jeju provinces) in South Korea were evaluated for CH newborn screening program. Central data were gathered in each city and collectively analyzed. Congenital hypothyroid subjects were followed for at least 2 years.

Newborn screening data were obtained from the SCL newborn metabolic screening program, which utilizes a 2-tier system including direct referrals of infants with markedly elevated TSH by pediatricians and secondary samples from those with only mild TSH elevation. To evaluate the efficacy of the newborn CH screening program in South Korea, we used set-up cut-off values and screening, diagnoses, and follow-up treatment data from a 14-year period (1991 to 2004).

2. Measurement of neonatal TSH

The Neonatal TSH ELISA kit (Bio-Rad) is an enzyme-linked immunoassay incorporating a biotinylated anti-TSH polyclonal antibody and an anti-TSH monoclonal antibody bound to the microwell. It is an amplified method of assessing TSH levels utilizing the biotin-streptavidin linkage to increase the strength of the generated signal. The polyclonal-monoclonal pair results in rapid incubation times and enhanced specificity. TSH is first eluted from the blood spot and simultaneously binds to the anti-TSH antibody on the microwell. During the next incubation period, a sandwich is formed between the added biotinylated antibody, the antibody presents on the microwell, and the eluted sample's TSH antigen. The plate is then washed to remove unbound material. Streptavidin-peroxidase is added which binds to the biotinylated antibody at numerous sites and the plate is washed again to remove unbound streptavidin-peroxidase. Then, TMB substrate solution is added. The substrate solution reacts with the enzyme to produce an amount of color directly proportionate to the amount of antigen present in the sample. From photometric absorbance readings, a standard curve was constructed and the amount of TSH in the sample quantified.

3. Measurement of neonatal free T4

The microplate neonatal free T4 kit (Bio-Rad) was used for the measurement of free T4 (fT4). The microplate Neonatal fT4 assay is a competitive EIA assay. Dried blood spot samples are eluted directly into anti-rabbit IgG antibody-coated microwells in a solution containing peroxidase labeled T4 and anti-T4 antibodies. After incubation, the wells are washed free of unbound labeled T4 and antibodies. The amount of bound fT4 present in the sample is determined by its reaction with the substrate and the resulting colored product. The competition between fT4 in the sample and enzyme-labeled T4, the color measured from the well is inversely proportional to the concentration of fT4 present in the sample.

4. Statistical analyses

All the analyses were performed in SPSS version 20 (SPSS Inc, Chicago, IL, USA). The results were presented as either frequencies or percentages. The Pearson's Chi-square test for categorical variables was used to compare the prevalence of transient CH between the genders. The Chi-square test was used to assess differences in the incidence of transient and permanent forms of CH, different etiologies, and gender ratios over time (probability values less than 0.05 were considered as significant).

5. Internal quality control

The acceptable range of the quality control material was set at an average of +/- 3 SDs based on 20 measurements. One plate included the quality control material and the TSH and free T4 of the associated specimens were measured using the methods described above. After confirming that the internal quality control result was within the acceptable range, the results of the experiment were reported.

6. Screening program performance

Evaluation of the performance of the South Korea-wide CH screening program using locally determined TSH and free T4 thresholds was performed. We found the sensitivity, specificity, and the positive predictive value (PPV) for the program as a whole.

Results

From January 1991 to March 2004, 671,805 infants underwent newborn screening (NBS) for CH in Seoul Clinical Laboratories (SCL). Of them, 159 CH-positive cases were identified (143 from the general newborn population, and 16 from repeat testing in low birth-weight infants). It is essential to evaluate the prevalence, regional distribution, and appropriate cut-off level for CH in Korean newborns.

The earliest procedures for assaying thyroxine (T4) and thyroid stimulating hormone (TSH) levels in dried blood spots on a mass scale were developed locally. The TSH assay was especially difficult to develop due to the questionable reliability of the assay. In addition to difficulties associated with the assays, there were no data on which to base protocols for the management of newly identified infants with CH.

In this study, we found the mean neonate TSH level to be 4.54 mIU/L (±2.89, SD) and that levels below 10 mIU/L were in the 94th percentile in the normal distribution of blood spots (Fig. 2). We thus determined the appropriate cut-off value for TSH level to be 20 mIU/L in normal blood spots, which ranked in the 95th percentile (Fig. 3A). The range of TSH levels from positive blood spots (including recall spots) was 30-250 mIU/L (Fig. 3B).

Further, our assays revealed the mean free T4

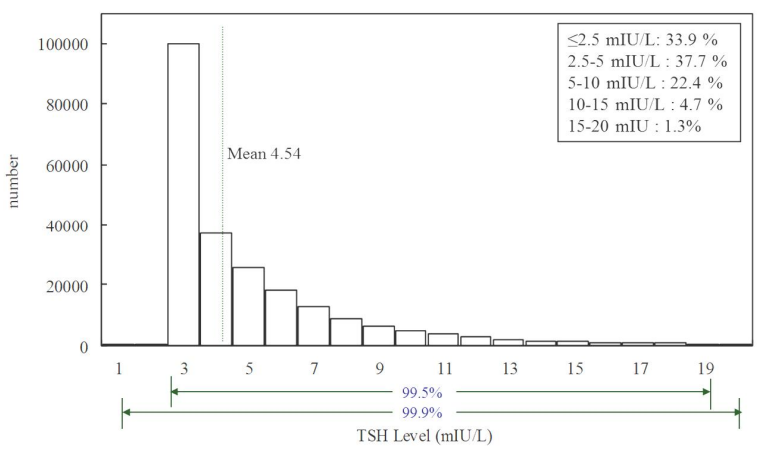


Fig. 2. Distribution of neonatal TSH level in normal blood spots.

level to be 2.02 ng/dL (±0.95, SD) and that levels of 0.8 ng/dL or below ranked in the 91st percentile (Fig. 4). We also determined the cut-off value for free T4 level was to be below 0.7 ng/dL in normal blood spots, which ranked in the 95th percentile (Fig. 5A). The free T4 level in positive blood spots (including recall spots) ranged from 0.1 to 0.7 ng/dL (Fig. 5B).

We next found 159 positive CH cases among patients in South Korea from 1991 to 2004; the distribution of the confirmed positive CH cases is shown in detail in Fig. 6.

The sensitivity and specificity of the TSH assay in this study were 100.0% and 99.7%, respectively, whereas the PPV was very low (10.8%). Further, the sensitivity and specificity of the free T4 assay were 100.0% and 98.5%, respectively, while PPV was also similarly low (10.8%) (Table 1).

The global CH frequency prior to 2,000 was around 1 in 3,000-1 in 5,600, which is very similar to the incidence reported in this study (Table 2). We also found that except for those in 1996 and 2003, the nationwide CH-positive rate

Table 1. Sensitivity, Specificity and Positive Predictive Value (PPV)

	TSH	Free T4
Sensitivity	100.0%	100.0%
Specificity	99.7%	98.5%
Positive Predictive Value	10.8%	3.9%

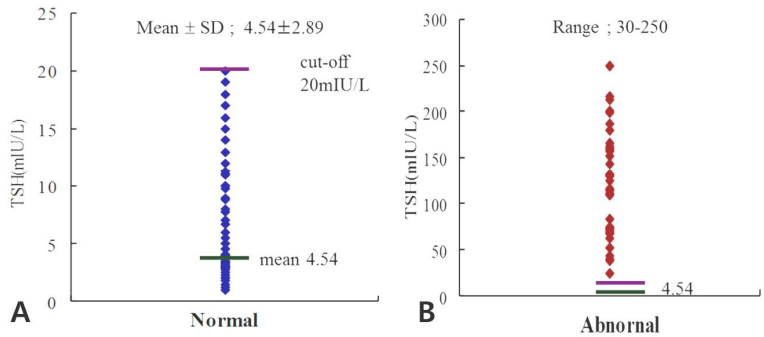


Fig. 3. Cut-off value from TSH level in normal (A) and abnormal blood spots (B).

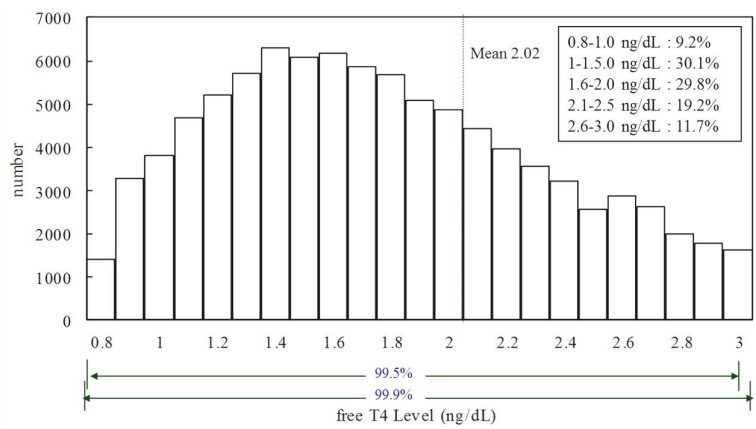


Fig. 4. Distribution of neonatal free T4 levels in normal recall spots.

Table 2. Global Frequency of Congenital Hypothyroidism

Country	South Korea* (SCL)	USA	Denmark	Australia	Switzerland
Frequency	1/4,558 (1/4,215)	1/5,000	1/4,580	1/5,600	1/3,000

*Source: Korea Institute for Health and Social Affairs.

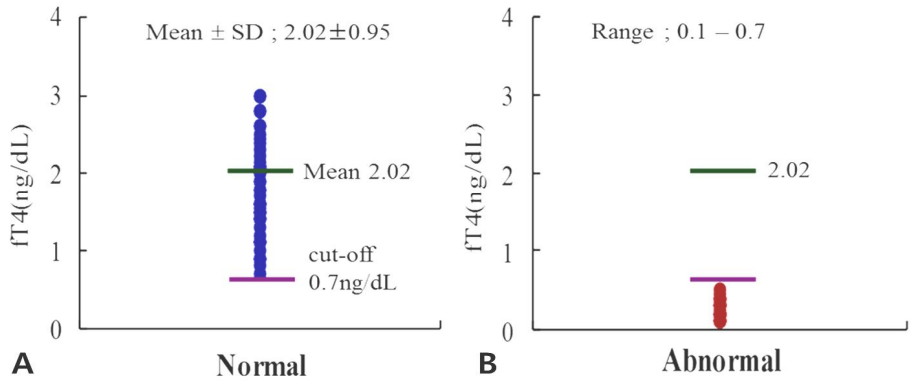


Fig. 5. Cut-off value for free T4 levels in normal (A) and abnormal blood spots (B).

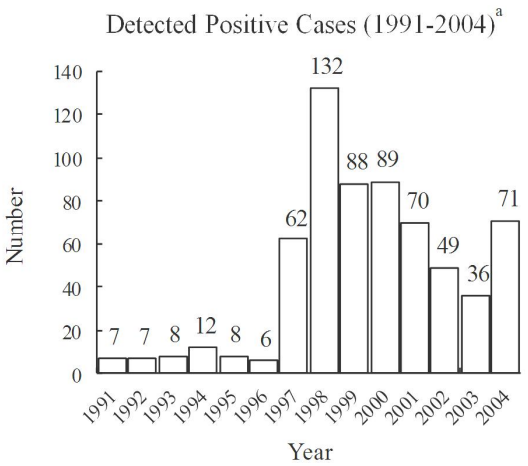


Fig. 6. Numbers of positive cases for congenital hypothyroidism patients in South Korea.

was very similar to the positive rates reported by SCL.

In 1998, 65% of newborns were screened for CH. Our study revealed that since 1998, the number of births has surpassed 330,000 per year, and the proportion of newborns screened for CH has increased sharply from 65% to 81% (Table 3).

We also observed a tendency for CH-positive rates to increase gradually relative to rates from overseas from the year 2000. This is a CH incidence regarding further study (Table 3).

In order to identify the regional distribution of CH incidence, we divided positive cases during the study period (1990-2000) into 6 metropolitan cities and 9 province areas based on their location. As expected, CH incidence was the highest in Seoul and Kyonggi Province, where the population and annual birth rate are relatively high. Ulsan, Daegu, and Gwangju, which have relatively small populations and low birth rates, ranked 6th, 7th, and 8th in the number of positive CH cases, respectively (Fig. 7).

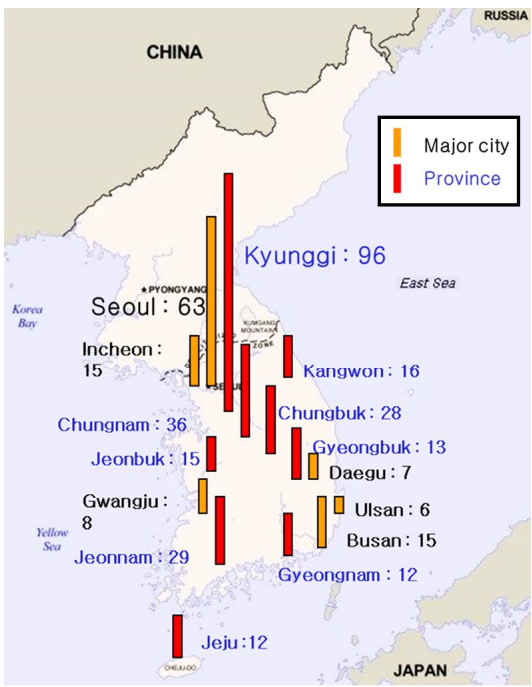
Discussion

The results of our 14-year retrospective study can be broken down into two main categories : cut-off levels (Fig. 3-5), and prevalence and efficacy of neonatal screening in South Korea (Fig. 6, 7 and Table 1). We detected 159 positive cases out of 671,805 screened newborns, with an asso-

Table 3. Incidence of Congenital Hypothyroidism from the Positive Cases Found by Newborn Screening (1991-2004)

Year	Nationwide Positive Case*		Incidence*	SCL Positive Case†	Incidence†
	Positive/No. of screened	% screened		Positive/No. of screened*	
1991	7/28,286	4	1/4,041	N.A.	N.A.
1992	7/20,372	2.8	1/2,910	N.A.	N.A.
1993	8/35,094	4.9	1/4,387	N.A.	N.A.
1994	12/51,045	7.1	1/4,254	N.A.	N.A.
1995	8/74,880	10.4	1/9,360	8/39,152	1/4,894
1996	6/62,542	9	1/10,423	7/36,813	1/5,259
1997	62/345,013	51.6	1/5,565	22/99,435	1/4,519
1998	132/416,115	65.6	1/3,152	24/92,686	1/3,862
1999	88/398,444	64.9	1/4,527	25/97,934	1/3,917
2000	89/407,981	64.3	1/4,584	19/77,868	1/4,098
2001	70/382,338	68.9	1/5,462	12/54,612	1/4,551
2002	49/346,176	70.3	1/4,382	17/69,690	1/4,099
2003	36/339,945	69.3	1/9,443	20/81,354	1/4,067
2004	71/386,889	81.8	1/5,449	5/22,261	1/4,452

Nationwide data, *Nationwide national newborn screening for PKU and CH carried out in South Korea.
†SCL newborn screening program data until March of 2004.
Abbreviations: SCL, Seoul clinical laboratory; N.A, not available.



*Source : Korea Institute for Health and Social Affairs.

Fig. 7. Regional distribution of congenital hypothyroidism patients in South Korea (1990-2000).

ciated prevalence of 1 in 4,225 (Table 2, 3). This nationwide study, which to our knowledge includes the largest cohort described to date, reports a CH incidence in South Korea similar to that reported by a study performed over a 14-year period from 1991 to 2004 (Table 2). This study was performed by a laboratory-only institution, and thus it was difficult to obtain clinical information. Due to these limitations, premature infants were repeatedly tested using recall blood spots.

In the past several decades[18,20-22], studies have evaluated TSH cut-off values for different ethnic groups worldwide, and attempted to reduce instances of false positives by lowering cut-off values. Thyroid dysfunction is common among very premature and low birth-weight infants[18]. Premature infants (<30 weeks gestation) commonly have thyroid dysfunction, with the majority of them being diagnosed with CH based on delayed TSH elevation that was missed by routine newborn screening[23].

Overseas newborn CH screening found that the frequency of CH had increased in recent years, although this was likely due to the improvement

of detection methods caused by various technological advances in the 2010s, rather than any real increase in CH incidence[12,17,20-23]. In the Korean population, however, the frequency of CH had remained constant for decades before the 2010s. Whether this difference is racial or technological should be investigated in future research.

Further studies are required to investigate the effect of CH with delayed TSH elevation on clinical and neurodevelopmental outcomes. The optimal TSH and free T4 cut-off values for both screening and replacement therapy also remain to be elucidated. In addition, it is important to determine whether appropriate cut off values are beneficial to the Korean population.

Notably, we found that the prevalence of CH in 1995, 1996, and 2003 was 1 in 9,360, 1 in 10,423, and 1 in 9,443, respectively; these results require a more thorough retrospective evaluation (Fig. 6, Table 3). The prevalence rates obtained from the confirmed cases of CH in SCL screening alone from 2001-2004 were 1 in 4,551 (2001), 1 in 4,099 (2002), 1 in 4,067 (2003), and 1 in 4,452 (2004). The prevalence of CH reported here is similar to that reported by overseas studies (Fig. 7, Table 2).

In our study, the positive predictive value (PPV) for the first screening test was 10.8% for TSH and 3.9% for free T4. After the second screening, the recall rate was reduced to 0.15% from 3.5% (data not shown) and PPV increased to 35.2%. Further, the median age at screening was 7 days, and for 92.3% of patients, treatment was initiated prior to reaching 40 days of age[3-9].

The newborn screening program has led to the successful early detection and treatment of neonates with CH, reducing the onset of severe neurodevelopment deficiencies resulting from late diagnosis[11].

Prior to the implementation of the NBS program, the incidence of CH diagnosis ranged from approximately 1 in 7000 to 1 in 10.000. In 1980, screening programs reported the incidence of CH as ranging from 1 in 3,000 to 1 in 4,000[7]. Harris and Pass reported that the incidence of CH as detected by NBS programs in the USA had nearly doubled in the past two decades, having increased from 1 in 3,985 to 1 in 2,273. Similar results were also reported from Western Australia (1 in 5,747 to 1 in 2,825), Italy (1 in 2,654 to 1 in 1,154), Greece (1 in 3,384 to 1 in 1,749), Northern England (1 in 2,702 to 1 in 1,078), and Brazil (1: 3,616 to 1:1,030)[7].

In the present study, we evaluated changes in CH incidence as detected by NBS from 1991-2004. Our results showed that the incidence of CH has remained relatively constant, being 1 in 4,041 from 1991-1995 and 1 in 4,452 from 2001-2004. Various factors have been reported to be associated with the increased incidence of CH as detected by NBS programs, including increases in the rates of premature and multiple births (e.g., twins, triplets) and heightened average maternal age[12,21,23]. We did not evaluate the influence of these factors on CH incidence in the present study; however, our results did not agree with the those of other studies that suggested that the incidence of CH is rising[7].

Most screening programs lower their TSH cut-off value to avoid missed cases. In Italy (2009), Corbetta et al. reported that the incidence of CH almost doubled when TSH cut-offs were lowered from 20 to 10 mIU/L[25]. They reported that 45.0 % of CH-positive newborns, including 8.5% of cases with thyroid dysgenesis, would have been missed using the previous cut-off values[25]. Permanent hypothyroidism was confirmed in 88.7% of CH newborns, while 1/3 of them had screened

TSH levels between 10 and 20 mU/L and would therefore have been missed if the cut off level had remained at 20 mU/L[25]. Similar findings were reported by Mangreli et al. in Greece (2010)[26]. Since the incidence of CH has increased, and screening programs should consider lowering the TSH cut-off threshold to 10 mIU/L.

The TSH cut-off value has been 20 mIU/L for 15 years in South Korea[19]. Extensive and careful research is required for the possibility of lowering the cut-off value for TSH level to avoid missed cases.

In this study, we found that the incidence of transient CH has significantly increased (data not shown). Further studies are needed to identify other factors associated with the increasing incidence of transient forms of CH in South Korea.

In South Korea, despite there being well-established protocols for the screening and detection of CH in newborns, stricter implementation of a structured system for CH monitoring and surveillance is needed to facilitate the management of patients and reduce the rates of missed follow-up cases. Determining and addressing the causes of high false positive rates must be prioritized at the 1st screening. A limitation of our study is the unknown frequency of cases diagnosed with transient hypothyroidism. Estimating the proportion of transient hypothyroidism over time would be useful for better interpreting this increase in the incidence of CH. Another limitation is that there were no data available on the ethnicity and consanguinity of patients throughout the study period.

Conclusion

In conclusion, our findings reveal that the overall incidence of CH in South Korea during the study period (14 years) was practically constant, with a slight increase in almost all etiological categories, and was not associated with other unidentified factors. Further studies are needed to identify other factors associated with the heightened incidence of CH observed in other countries of the world. The outcomes of patients treated early (within 2 weeks of diagnosis) were generally favorable. Neonatal screening for TSH and free T4 using the ELISA method was an effective method of early diagnosis and treatment for patients. The association between lowering TSH cut-offs and CH prevalence requires further study.

요 약

목적: 선천성 갑상선기능저하증(Congenital hypothyroidism, CH)은 전세계적으로 출생아 3,000-4,000명 당 1명의 빈도로 발생하는 신생아기에 가장 흔한 내분비질환이다. 본 연구는 1991년부터 2004년까지 남한의 선천성 갑상선기능저하증 발생률의 빈도 및 현재까지 적용되어온 광범위한 신생아 선별검사의 검사방법과 결과를 재평가하기 위해 수행되었다.

방법: 서울 지역에 위치하지만 전국적인 지사를 운영하고 있는 검사기관 서울의과학 연구소(SCL)에서는 서울 외 6개 광역시(부산, 인천, 대구, 대전, 광주, 울산)와 9개도(경기, 강원, 충북, 충남, 전북, 전남, 경북, 경남, 제주)로 한국을 15개 지역으로 분류하였다. 15개 지역으로부터 신생아의 채혈지를 수집하여 서울본원에서 수집하여, TSH 및 유리 T4를 ELISA 검사법으로 신생아선별검사를 수행하였다. SCL 데이터 및 복지부에 보고된 전국적인 양성자수를 검토하였다. 선천성갑상선기능저하증에 대한 신생아선별 검사법의 cut-off 치는 신생아 갑상선자극호르몬(TSH) 측정을 위해서는 20 mIU/L를 유리 T4의 검사를 위해서는 0.8 ng/dL 이하를 사용하였다. TSH 및 유리 T4 ELISA 분석에 기초한 선천성 갑상선기능저하증에 대해 1차 선별검사에서 선천성 갑상선기능저하증 양성은 신생아 671,805명 중 신생아 159명에서 발견되었으며, 이의 발병빈도는 4,225명 중 1명으로 추정되었다.

결과: TSH 분석에서 cut-off 20 mIU/ L를 사용했을 때 민감도, 특이도 및 양성 측도(PPV)는 각각 100.0 %, 99.7% 및 10.8%였다. 유리 T4 분석을 위해 0.8 ng/dL cut-off를 사용했을 때 민감도, 특이도 및 양성 예측도는 각각 100.0%, 98.5% 및 3.9%였다.

결론: NBS를 통해 얻은 CH의 발병빈도는 2004년 이전에 해외의 여러 국가에서 보고 된 발병빈도와 비교할 만큼 유사하였다.

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Conflicts of Interest

The authors have nothing to declare.

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