IODINE DEFICIENCY DISORDERS (IDD) : THE EVALUATION OF ITS ELIMINATION IN KHAMCHA-I , MUKDAHARN

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(RADIOLOGICAL SCIENCE)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2000

ISBN 974-664-155-7

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was submitted to the Faculty of Graduate Studies, Mahidol University
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ACKNOWLEDGEMENT

My sincere appreciation is express to Professor Romsai Suwannik for his regarding advice, helpful comments and constructive criticism for my thesis. I am extremely thankful to Professor Rudee Pleehachinda for her being the chairman of my thesis candidate. Specially thank to major adviser, Associate Professor Chaveevan Pattanachak for her kindness guidance, regarding encouragement and intensive supervision throughout this study that enabled me to carry out this thesis successfully.

I would like to thank Associate Professor Supong Pattanachak for allowing me to utilize computerized work in Nuclear Medicine Building. Thank are also extends to all members of Nuclear Medicine Division, Siriraj Hospital especially Ms. Siriporn Chongchirasiri, Mr. Boontham Amorngitticharoen for their encouragement in Chemistry laboratory. In addition, thank are also extends to Ms. Nucharee Putraseranee for her helpful about photographic and computerized application.

I also thank to Dr. Chuensuk Juntaramaeteegul for her kind provision of ultrasonic machine and excellent performs in ultrasonic examination for my calibration. In addition my thesis was supported in part by the thesis grants, Faculty of Graduate Studies, Mahidol University.

Finally, I owe unending gratitude to my dear parents for their love, encouragement and will for me. I can not reach to this goal without them.

Prathan Wongtala

4037375 SIRS/M: MAJOR: RADIOLOGICAL SCIENCE; M.Sc. (RADIOLOGICAL SCIENCE)
KEY WORDS : IODINE DEFICIENCY DISORDERS (IDD) / EVALUATION/ ELIMINATION

PRATHAN WONGTALA: IODINE DEFICIENCY DISORDERS (IDD); THE EVALUATION OF ITS ELIMINATION IN KHAMCHA-I, MUKDAHARN. THESIS ADVISORS : CHAVEEVAN PATTANACHAK, M.Sc., ROMSAI SUWANIK, M.D. 75 p. ISBN 974-664-155-7

The evaluation of iodine deficiency disorders (IDD) using WHO/UNICEF/ICCIDD indicators was performed in 543 primary schoolchildren of both sexes and aged 6-14 years in Khamcha-I District, Mukdaharn Province. In pre-test the prevalence of goiter, an indicator of IDD was checked and found to be 4.58 % by palpation and 19.08% by ultrasound in the placebo group (Namteung, n=131). The prevalence of goiter was found to be 20.0% by palpation and 44.71 % by ultrasound in the treated group (Nong-ean-dong, n=85). The treated group showed moderate sign of IDD by palpation, but severe signs by ultrasound. The placebo group showed no signs of IDD by palpation and mild signs by ultrasound. The discrepancy between ultrasound and palpation was 14.5 % in the placebo group and 24.7% in the treated group. The percent of serum TSH at > 10 mIU/L showed no IDD problem in both the placebo group (0%, n=68) and the treated group (0.07%, n=139). The median urinary iodine level showed mild IDD in both groups which were 62.3 and 56.6 μg/L in the placebo group (n=87) and the treated group (n=117) respectively.

One year after receiving iodated water (150 μgI/day) in the treated group and water without iodine fortification (median iodine= 25.43 μg/L) in the placebo group, the incidence of goiter by ultrasound was decreased to 8.33% and to 9.73% respectively. When compared with palpation, the incidence in the treated group was also decreased to 13.09% but slightly increased to 5.31% in the placebo group. A discrepancy between ultrasound and palpation was also found 4.42% in the placebo group and 4.76% in the treated group. It is indicated that palpation is relatively inaccurate for assessing the prevalence of goiter in the iodine deficiency area. In contrary, it can be applied to assess the prevalence of goiter in iodine-replete areas (goiter prevalence <5%). Serum T_3, T_4 and TSH of pre-and post-test in both groups were within the normal limits. The median urinary iodine levels in the treated group were higher than normal value of 126.6 μg/L after receiving iodated water for 3, 6, 9 and 12 months. In the placebo group, median urinary iodine was also increased from the pre-test but lower than the normal value.

To eliminate IDD in primary schoolchildren, iodine prophylaxis program should continue. Monitoring and evaluation of IDD by thyroid gland palpation and urinary iodine excretion should be done once a year.

4037375 SIRS/M : สาขาวิชา : วิทยาศาสตร์รังสี; วท.ม. (วิทยาศาสตร์รังสี)

ประธาน วงศ์ตาหล้า : การประเมินการควบคุมภาวะผิดปกติจากการขาดธาตุไอโอดีนที่อำเภอคำชะอี จังหวัดมุกดาหาร (IODINE DEFICIENCY DISORDERS (IDD): THE EVALUATION OF ITS ELIMINATION IN KHAMCHA-I, MUKDAHARN). คณะกรรมการควบคุมวิทยานิพนธ์ : ฉวีวรรณ พัฒนจักร M.Sc., ร่มไทร สุวรรณิก M.D. 75 หน้า ISBN 974-664-155-7

ได้ทำการศึกษาภาวะโรคขาดสารไอโอดีน ในเด็กนักเรียนชั้นประถมศึกษา อายุ 6-14 ปี จำนวน 543 คน ที่ จ.มุกดาหาร โดยนำข้อบ่งชี้ของ WHO/UNICEF/ICCIDD มาพิจารณา พบว่าในกลุ่มควบคุม (ร.ร.น้ำเที่ยง, n=131) มีอุบัติการคอพอกด้วยวิธีคลำคอเท่ากับ 4.58%ซึ่งไม่เป็นปัญหาของ IDD ส่วนวิธีอัลตราซาวนด์เป็น Mild (19.08%) ในกลุ่มทดลอง (ร.ร.หนองเอียนดง, n=85) มีอุบัติการคอพอกด้วยวิธีคลำคอเป็น Moderate (20.0%) วิธีอัลตราซาวนด์เป็น Severe (44.71%) ความแตกต่างระหว่างวิธีอัลตราซาวนด์และคลำคอในกลุ่มควบคุมเท่ากับ 14.5% กลุ่มทดลอง เท่ากับ 24.7% เปอร์เซนต์ของระดับซีรั่ม TSH > 10 mIU/L ในกลุ่มควบคุมมี 0% (n=68) และกลุ่มทดลองมี 0.07% (n=139) ทั้ง 2 กลุ่มไม่มีปัญหาของ IDD ค่ากลางของไอโอดีนในปัสสาวะของนักเรียนกลุ่มควบคุม (n=87) 62.3 μg/L และกลุ่มทดลอง (n=117 คน) 56.6 μg/L แสดงว่าเป็น Mild ทั้ง 2 กลุ่ม

ภายหลังจากที่นักเรียนกลุ่มทดลองได้รับน้ำไอโอดีนทุกวัน วันละ 150 μg และนักเรียนกลุ่มควบคุมได้รับน้ำที่ไม่ได้เสริมไอโอดีน (ค่ากลางของไอโอดีน 25.43 μg/L)เป็นเวลาหนึ่งปี พบว่ากลุ่มทดลองและกลุ่มควบคุมมีอุบัติการคอพอกโดยวิธีอัลตราซาวนด์ลดลง เป็น 8.33% และ 9.73% เมื่อเปรียบเทียบกับการคลำคอพบว่ากลุ่มทดลองจะลดลงเป็น 13.09% แต่กลุ่มควบคุมจะเพิ่มขึ้นเล็กน้อยเป็น 5.31% ความแตกต่างระหว่างวิธีอัลตราซาวนด์และการคลำคอเท่ากับ 4.42% ในกลุ่มควบคุมและ 4.76% ในกลุ่มทดสอบ อุบัติการคอพอกโดยวิธีคลำคอจะให้ผลคลาดเคลื่อนจากความเป็นจริงในถิ่นที่ขาดสารไอโอดีนแต่จะให้ผลใกล้เคียงกับวิธีอัลตราซาวนด์ในถิ่นที่มีอุบัติการคอพอกน้อยกว่า 5% ส่วนซีรั่ม T_3,T_4,TSH ก่อนและภายหลังการประเมินของทั้งสองกลุ่มมีค่าอยู่ในระดับปกติ ค่ากลางของระดับไอโอดีนในปัสสาวะในกลุ่มทดสอบมีค่าสูงกว่าเด็กปกติ ภายหลังจากได้รับน้ำไอโอดีน 3, 6, 9 และ 12 เดือน ในกลุ่มควบคุมไอโอดีนในปัสสาวะมีค่าสูงขึ้นเช่นกันแต่ก็ยังต่ำกว่าเด็กปกติ

เพื่อที่จะกำจัดโรคขาดสารไอโอดีนในเด็กนักเรียนประถมศึกษา ควรจัดให้มีกิจกรรมดังกล่าวข้างต้นต่อไป โดยมี monitoring และ evaluation ด้วยการคลำคอ และ ตรวจหาไอโอดีนในปัสสาวะทุกปี

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LIST OF ABBREVIATIONS

Ab	antibody
Ag	antigen
As^{5+}	arsenic
As^{3+}	arsenite
ANS	8-anilino-1-napthalene sulphonic acid
B	bound form of standard or unknown
Bo	bound form of zero standard
BSA	bovine serum albumin
BKK	Bangkok
cpm	count per minute
cm	centimetre
^{o}C	celsius degree
Ce^{4+}	ceric
Ce^{5+}	cerous
cf	correction factor
χ^2	chi-square
CDI	1,1' carbonyldiimidazole
DIT	diiodothyronine
DASS	donkey anti sheep serum second antibody
dl	decilitre
FT_4	free thyroxin
Fig	figure
g	gram
HFS	hormone free serum
hr	hour
L	litre
IRMA	immunoradiometric assay
IDD	iodine deficiency disorders
I^-	iodide
I	iodine ion
μ	micro
μg	microgram
μgI_2	micragram iodine
μl	microlitre
M	molar
min	minute
mg	milligram
mIU	milli international unit
MIT	monoiodothyronine
MHz	megahertz
ml	millilitre
mAb	monoclonal antibody

LIST OF ABBREVIATIONS (continue)

MP	magnetic particle
n	nano
ng	nanogram
nm	nanometer
NSB	non specific binding
PEG	polyethylene glycol
P 97	97 percentile
r	correlation coeffcient
RIA	radioimmuno assay
ROC	receiver-operating characteristic
sec	second
SD	standard deviation
TSH	thyroid stimulating hormone
TRH	thyrotropin releasing hormone
T_3	triiodothyronine
T_4	tetraiodothyronine
Tg	thyroglobulin
TCH	transient congenital hypothyroidism
yrs	years
-yr-old	year old

CHAPTER I

INTRODUCTION

The term of Iodine Deficiency Disorders (IDD) was introduced in 1983 due to the wide spectrum of effects of iodine deficiency on growth and development. In the past, the term 'goiter' has been used for many years to describe the effect of iodine deficiency. The earlier history of the development of knowledge of endemic goiter and cretinism- the classical features of iodine deficiency has been well reviewed by Kelly and Snedden and by Langer (1). The function of iodine is to constitute on essential substrate for synthesis of thyroid hormones tetraiodothyronine or thyroxin (T_4) and triiodothyronine (T_3). Thyroid hormones play a decisive role in the metabolism of all cells of organism and in process of early growth and development of most organs, especially of the brain. IDD includes: goiter at all ages; endemic cretinism, characterized most commonly by mental deficiency, deaf mutism, and apastic diplegia, and lesser degree of neurological defect related to fetal iodine deficiency: impaired mental function in children and adults with goiter, associated with reduced level of T_4; and increased stillbirths, and perinatal and infant mortality (2,3,4).

Anatomy of the thyroid gland

The word thyroid derives etymologically from the Greek word Thyos, meaning oblong shield. Actually, this term is not quite appropriate, because the thyroid gland does not resemble an actual shield but is so name because of its close proximity to the

thyroid cartilage (5). The thyroid gland is the large endocrine gland and can be recognized in the human embryo by the end of the first month after conception when embryo is 3.5-4 mm in the length. In humans weighting about 20 g in an adult (6,7). The normal adult thyroid consists of two lateral lobes (8). The lobes are about 5 cm long, 3 cm across at the wide part, and 2 cm thick at the broader lower pole (9). There are connected by the isthmus, which is a band of variable size lying close to the ventral aspect of the trachea, covering the 2 nd, 3 rd, and 4 th tracheal rings in about 40 % of cases a pyramidal lobes extends upward from the upper margin of the isthmus toward the hyoid bone. The two lobes of the gland embrace the ventral and lateral aspects of the trachea, usually just below the cricoid cartilage (8). The thyroid gland has a rich blood supply and the right lobe more vascular and often is larger. The artery arises from the common or external carotid artery, the inferior thyroid artery from the thyrocervical trunk of the subclavian artery, and the small thyroid artery from the brachiocephalic artery at the aortic arch. Venous drainage is via multiple surface veins coalescing into superior, lateral, and inferior thyroid veines. The blood flow to thyroid gland is about 5 ml/g/min (10, 11).

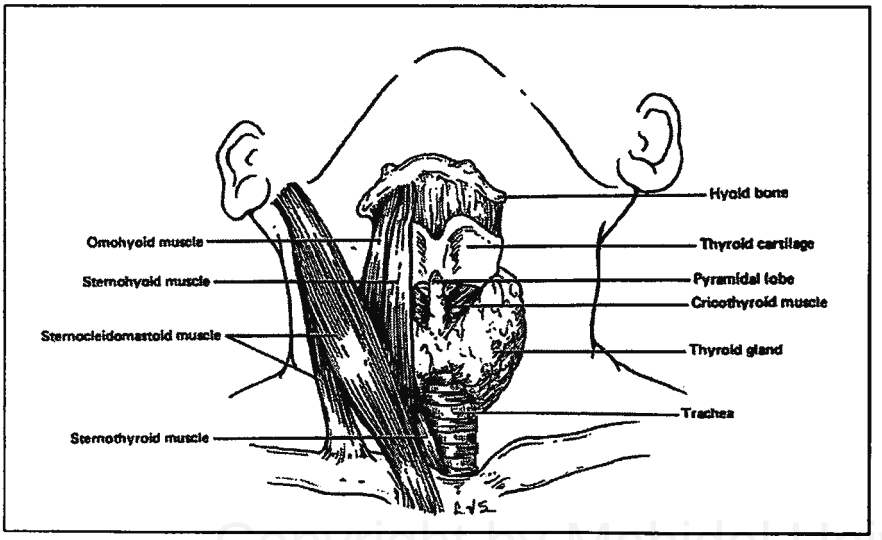


Fig.1. The thyroid gland (12).

Thyroid hormone synthesis (2)

Whatever the from of ingested inorganic iodine, it is eventually converted to iodide and absorbed as such into the circulation. Dietary iodide in the blood stream is brought to thyroid follicular cell by highly efficient transport system, which is called 'the iodine pump'. This mechanism is regulated by the thyroid-stimulating hormone (TSH) or thyrotrophin, which is released from the pituitary to regulate thyroid secretion. The iodide is released by the thyroid cells into the colloid follicle phase between the cells, where it is oxidized by hydrogen peroxide from the thyroid peroxidase system. It then combines with tyrosine in the thyroglobulin to form monoiodothyrosine (MIT) and diiodothyrosine (DIT). The oxidation process then continues with the coupling of MIT and DIT to form the thyroxine (T_4) and triiodothyronine (T_3).

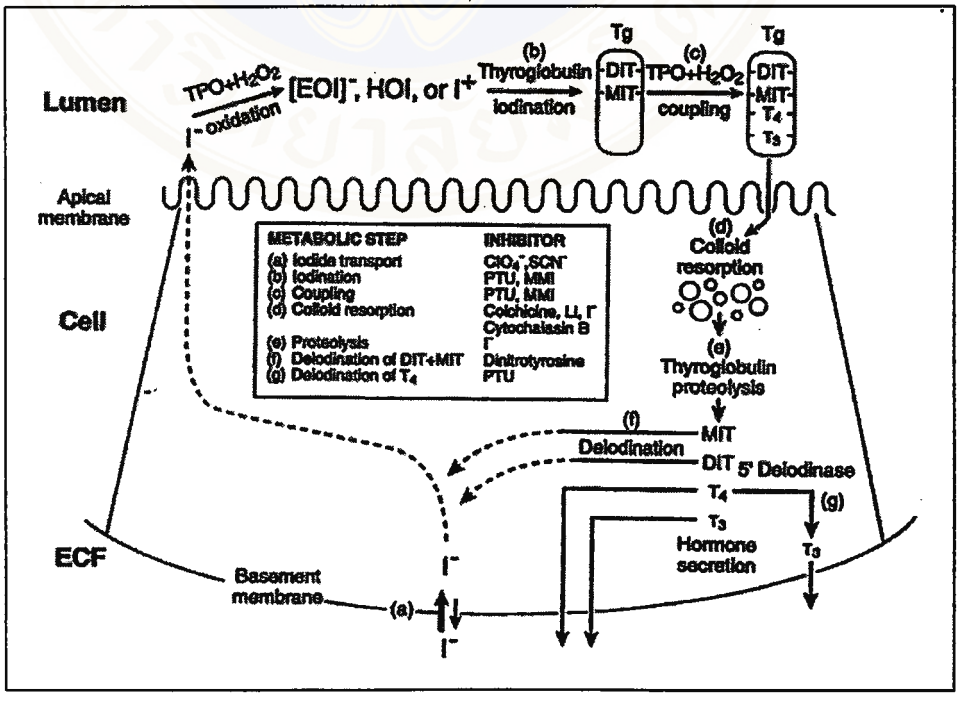


Fig. 2. Thyroid hormones synthesis (13).

Finally, the iodized thyroglobulin, including the iodized amino acid, is absorbed back into the thyroid cell by a process called 'pinocytosis'. It is then exposed to proteolytic enzymes, which break it down to release the T_4 and T_3. The unused iodotyrosines are conserved for incorporation into a subsequent cycle of the biosynthetic process, which state of thyroid stimulation. This conservation process may not be able to keep pace with the production of free iodotyrosines. These may leak into the circulation but have no apparent biological effect.

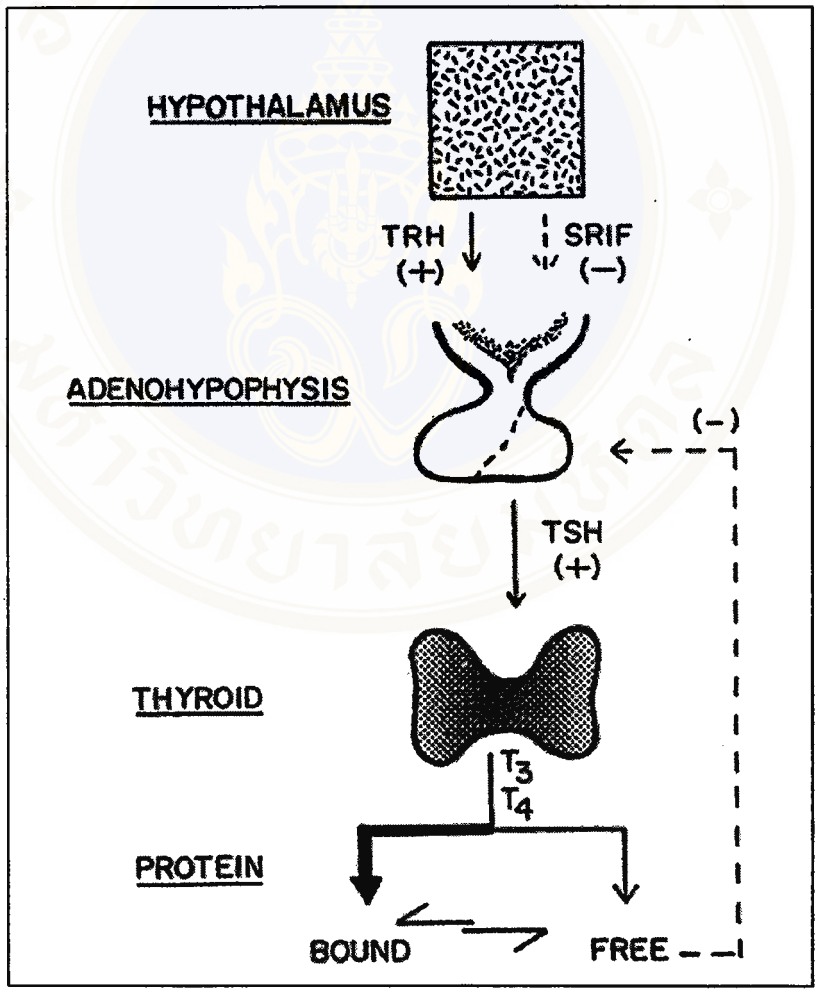


Fig. 3. The relationship among hypothalamus, pituitary, thyroid, and other tissue (7).

The control of TSH secretion by a 'feed back' mechanism related closely to the level of T_4 in the blood. As the blood T_4 falls, the pituitary TSH secretion rises to increase thyroid activity and the output of T_4 into the circulation. TSH secretion is also under the control of the brain through the thyrotophin-releasing hormone (TRH), which is released from the hypothalamus, a small region at the base of the brain (close to the pituitary, which is very important for the control of all the pituitary hormones. TRH is released into the pituitary portal system from which it goes direct to the pituitary. There, it influences the synthesis and release of TSH, which is produced by special cells in the anterior lobe of the pituitary (14).

Iodine deficiency (14,15)

1. The consequences of iodine deficiency.

A trace element of iodine occurs in the human body in only small amount (15-20 mg) and the essential requirement for normal growth are only 100-150 μg/day (0.1-0.15 mg). The deficiency of iodine has several important health consequences as follow:

1.1 Goiter

The best known effect of iodine deficiency is endemic goiter. Goiter is a swelling of the thyroid gland. Iodine deficiency is the major primary etiological factor in endemic goiter. People with iodine deficiency have goiters because they do not make enough thyroid hormone. The pituitary detects the low thyroid hormone levels in the blood, and makes more of its controlling hormones (TSH), which makes the thyroid work harder to produce more thyroid hormone. This

increased TSH stimulation is a normal adaptation but it produces a goiter, particularly if stimulation becomes chronic because of continued iodine deficiency. The goiter is a sign that the body is trying to compensate for lack of iodine. Other causes of goiter exist, but increases TSH stimulation is responsible for the goiter in area of iodine deficiency. Sometimes goiters compress the windpipe and produce chocking. They can also interfere with swallowing. In other cases the goiter may not give its owner symptoms, but it is still an indication of iodine deficiency and may be accompanied by more serious manifestations.

1.2 Hypothyroidism

This word means that the body does not receive enough thyroids hormone. Low levels of thyroid hormones in the blood detect hypothyroidism. Hypothyroidism produces sluggishness, sleepiness, dry skin, cold intolerance, and constipation. In very young children, it produces not only these features but also mental and growth retardation, because the thyroid hormones are essential for normal development of the brain and nervous system. Sometimes the mental retardation is very severe; at the other times it is mild and may not be recognized unless specifically looked for. Hypothyroidism in newborn is particularly serious, because the mental retardation it produces cannot be corrected it is permanent. This condition is called ' neonatal hypothyroidism'.

1.3 Cretinism

This term refers to the very severe consequences of hypothyroidism occurring during fetal or neonatal life. Cretins have severe irreversible short stature, and retarded development of the musculo-skeletal

system. Some cretins also have goiter and obvious hypothyroidism, but others do not frequently iodine deficiency produces intellectual or developmental retardation that is not severe enough to be classified as cretinism. Some writer to describe these conditions uses the term 'subcretin'.

1.4 Reproductive failure

Woman in severely iodine deficient areas has moremiscarriages, stillbirths, and other problems of pregnancy and reproduction than iodine-sufficient woman. It is difficult to estimate how often these complications occur, but they are probably much more frequent than generally realized. Continued miscarriages and fetal wastage decrease the fertility of a population and endanger the health of women.

1.5 Childhood mortality

Iodine deficiency kills children. Their defense against infections and other nutritional problems are lower than those of children in iodine-sufficient. For example, when iodine was given to pregnant women, their new born children had higher birth weight and a double survival rate when compared with children of mothers who had not received iodine.

1.6 Socioeconomic retardation

Iodine deficiency affects the socioeconomic development of a community in two ways. First, the people are mentally slower and less vigorous. They are harder to educate and harder to motivate, and thus they are less productive in their work. Also, iodine deficiency produces more handicapped individuals who depend on others for their care, thus diverting community resources. Secondly, in most of these areas agriculture is the most important economic activity, and domestic

animals suffer from iodine deficiency in much the same way that people do. Thus, domestic animals will be smaller and produce less meat, eggs, and wool. They also have more abortions and are frequently sterile.

2. Geographical distribution of iodine deficiency

Iodine occur in fairly constant amounts in ocean water but is distributed very unevenly in the earths crust. Inland region far from the ocean has the greatest risk of iodine deficiency. Some of the most severe iodine deficiency occur in relatively young mountainous area, where iodine in the soil has been washed away by rain and glaciers. However, iodine deficiency is not confined to high mountains, and also occur in large parts. It has also been associated with area exposed to frequent flooding and in large river delta.

3. Epidemiological measurement of IDD.

Several methods are employed in quantifying the extent and severity of IDD. These methods are as follow:

1. Palpation of the thyroid size.
2. Urinary iodine excretion.
3. Thyroid volume by ultrasound.
4. Thyroglobulin concentration in serum.
5. Thyrotropin (TSH) concentration in serum.
6. Thyroid hormones (thyroxin and triiodothyronine) in serum.
7. Radioiodine uptake.
8. Prevalence of cretinism.

3.1 Palpation of the thyroid size.

For examination of school children or adults, the examiner sits facing the subject, places his two thumbs on either side of the subject's windpipe several centimeter below the notch of thyroid cartilage (the 'Adam's apple) and rolls his thumbs gently over the thyroid, which lies next to the windpipe. This technique is call ' palpation'. The first decision should be whether or not the subject has a goiter. If each lobe of the thyroid is smaller than the part of the subject's thumb beyond the last joint (the terminal phalanx), the thyroid is classified as Grade 0, no goiter. If each lobe is not visible but palpable larger than the terminal phalanx of the subject's thumb, the goiter is classified as Grade 1. If it can be seen with the subject looking straight ahead, it is called Grade 2.

Table 1. Classification of goiter from WHO/ UNICEF/ ICCIDD Consultation on Indicators for Assessing Iodine Deficiency Disorders and control Programs: Geneva. 1992.

Grade 0	No palpable or visible goiter
Grade 1	A mass in the neck that is consistent with an enlarge thyroid That is palpable but not visible when the neck is in the normal position. It moves upward in the neck as the subject swallows. Nodular alteration(s) can occur even when the thyroid is not enlarged.
Grade 2	A swelling in the neck that is visible when the neck is in a normal position and is consistent with an enlarged thyroid. When the neck is palpated.

3.2 Urinary iodine excretion.

Approximately 90% of ingested iodine are excreted in the urine. Measurement of iodine in the urine provides a good index of the iodine taken. Since

the absolute minimum daily iodine requirement is about 50 μg, a urinary iodine level of less than 50 μg per day means iodine deficiency. In fact, some degree of iodine deficiency may exist even when the urinary iodine excretion is as high as 100 μg per day. In the field its almost impossible to collect all the urine passed by a subject during a 24-hour period. Instead, examiners must rely on casual urine samples, which are easier to obtain. Two general approaches have been used to relate the iodine content of a casual urine sample to 24 hour value. One approach relates urinary iodine to urinary creatinine but has not proved to be helpful because of variation in creatinine excretion with nutritional status so that this ratio is no longer used (16). The other approach is to simply measure the concentration of iodine in the urine, as μg iodine per 100 ml urine (μg/dl). While subjects will vary in the concentration of their urine, depending on how much liquid they have been drinking. This variation will tend to even out among samples from many subjects. For this reason that samples be obtained from at least 40 subjects to determine the median concentration of urinary iodine in a given region. Most experts prefer the expression of urinary iodine in this latter method (as a concentration), because it is simpler and has usually proven more reliable than relating creatinine. The detail of the laboratory procedure for measuring iodine in urine are given in the technical manual ' Laboratory Testing for Iodine Deficiency' being performed for this series. Urine sample will be acid digested at high temperature (~175 °C) to remove other substance, such as nitrite, thiocyanate or ferrous iron, which are interfere chemical reaction. Iodine in urine is normally in the form iodine ion $(I)^-$. Iodine ion will act as catalyst for reduction ceric ion (Ce^{4+}) to cerous ion (Ce^{3+}),

oxidation of arsenite from (As^{3+}) to arsenic (As^{5+}). The oxidation-reduction is called Sandell- Kolthoff reaction.

$$2\,Ce^{4+}\,(Ceric) + 2\,I \longrightarrow 2\,Ce^{3+}\,(Cerous) + I_2$$

$$I_2 + As^{3+}\,(Arsenite) \longrightarrow As^{5+}\,(Arsenic) + 2\,I^-$$

Ceric ion (Ce^{4+}) is yellow while cerous ion (Ce^{3+}) is Colourless.

While oxidation, yellow will be slowly disappeared which will be the exact rate of iodine ion (I^-) concentration in accelerate oxidation. Then the color changing will be measured by spectrophotometer. The equipment is frequently available in routine chemistry laboratories. One experienced technician can perform about 20-50 analyses per day, including standards for comparison. The advantages of assessment by this method are that the method is entirely objective, it is non-invasive, and it provides information on the one factor that can be addressed directly, i.e., iodine supply to the individual. The excretion of iodine indicates the recent but not precisely the immediate intake of iodine. Samples need not be processed immediately; they can be held until they are returned to the laboratory, or they can be shipped to a distant processing point. The problem with iodine excretion is that it requires a laboratory geared to providing accurate determinations, and it incurs some expense. Recent developments have much simplified measurements of iodine in the urine, making it easier and within the limitations of a modest budget. The results of a survey must be interpreted. In general, if the median concentration is below 10 μg/dl there is a strong suspicion of IDD in the region surveyed. If the value is below 5 μg/dl there is almost

surely IDD, and if it is below 2.0 μg/dl, the problem is a serious one demanding immediate attention.

3.3 Thyroid volume by ultrasonography

Ultrasonography, a means of obtaining an image of thyroid size by ultrasonic wave can provide a more accurate assessment of thyroid size. The procedure is not invasive, and can be used to measure several hundred subjects in a day. Its accuracy diminishes when the gland is quite large, but in such instances precise volume is not important for epidemiological purpose. The technique can be easily learnt within a few days. The use of ultrasonography, by providing an objective measure of thyroid volume, may in some case show that concern for IDD is unwarranted, and accordingly an expensive program of prevention could be avoided. The disadvantages of ultrasonography are a requirement for training, The cost of the instrument, and the problem of transport from center to survey site. Ultrasonography is rapidly replacing palpation, and has thrown doubt on the validity of many older surveys.

3.4 Thyroglobulin concentration in the blood

The thyroid gland leaks thyroglobulin into the blood at the rate roughly dependent on its size. Recent studies have indicated that measurement in serum can grossly mirror the size of the thyroid in region where IDD is found. Increased the concentrations of serum thyroglobulin have been found in goitrous subjects, and especially in those with large goiters. This reflects to some extent the degree of thyroid stimulation by TSH and is also related to the development of autonomous thyroid function. The advantage of a thyroglobulin assay is its objectivity

and reliability. The method has the advantages that it require invasion (a blood sample), and that the measurement, being a immunoradiometric assay, necessitates special laboratory expertise, special equipment, and a supply of the radio-labeled antibody. It appears to be a promising and potentially useful indicator of IDD, but has as yet not been thoroughly validated for epidemiological assessment.

3.5 Thyrotropin concentration in the blood

Thyroid stimulating hormone (TSH) is secreted by the pituitary and controls the thyroid gland function. Newly introduced ultrasensitive methods for measuring the concentration of thyrotropic hormone in the blood permit accurate assays within and between normal and slightly elevated values. Since thyroid enlargement in IDD is presumed to depend on response in TSH to a deficiency of iodine, hypothetically serum TSH concentration should reflect iodine deficiency, and perhaps would do so more accurately and immediately than goiter size itself. TSH concentration might be an immediate indicator of hormone production, which in turn should reflect iodine nutrition. Several factors blur this simplistic view. Because the thyroid has an enormous capacity to store iodine and buffer the hormone secretion rate, serum TSH value may not reflect recent dietary iodine supply, or deficits more remotely in time. Indeed, in most endemia, measurements of serum TSH concentrations of subjects with endemic goiter, or of those living in endemic goiter area, have been well within normal limits. An alternative use of TSH as a signal of IDD is in the newborn. This procedure is now widly used in developed countries to screen for the occurrence of congenital hypothyroidism. This condition is associated with a defect in the production of thyroid hormones due to the absence of the thyroid,

a small or misplaced thyroid, or a defect in the biochemical machinary in the thyroid for the production of thyroid hormones. If there is adequate iodine uptake, congenital hypothyroidism occurs at rate about 1 in 4,000 births (0.025%). As already, if iodine intake is inadequate then the rate may increase to 1 % of birth, which indicates a massive threat to brain development. This measurement then can be used as a population indicator of IDD quite apart from its used as a screening measure for individuals. On the other hand, if there is a very low rate of detection of neonatal hypothyroidism, then this can be cited as avoidance that the elimination of IDD has been achieved. These data are readily available in many developed countries where universal screening of newborn is well established.

3.6 Thyroid hormones (Thyroxine and Triiodothyronine)

The objective of the thyroid system is to supply to the body an appropriate amount of the thyroid hormone, of which thyroxine represents quantitatively the main form and triiodothyronine the most active form. In iodine deficiency there is a preferential production of the less iodinated hormone, with a change in the ratio between serum T_3 and T_4. In regions of endemic goiter, the measurement of the amount of hormone in the blood might be the ultimate tool for measuring IDD, except for several considerations. If iodine is given prophylactically to a community with IDD there is generally a net rise in mean serum thyroxine concentration. This presumably indicates that the population was deficient in iodine, but most often this change occurs within the accepted limits of normal, and few individuals have values below the accepted lower limit of the normal. Serum concentrations of triiodothyronine are even less definitive in this regard.

3.7 Radioactive 131- Iodine uptake

Early studies on the pathophysiology of endemic goiter used radio-iodine uptake to demonstrate the ambient lack of dietary iodine as reflected in the avidity of the deprived thyroid for radio-iodine. An elevate radio-iodine uptake shows that at the moment of testing, the thyroid lacks iodine. It may days or weeks of dialy supplements of iodine to restore the uptake to a level consistent with normal gland function. The radio-iodine uptake test would remain a first-line tool for measuring the need for iodine if it were not for certain disadvantages. Such disadvantages are: its use requires rather expensive and cumbersome apparatus, trained observers, and a supply of the radioisotope. The test also entails a small radiation exposure to both subject and investigator.

3.8 Prevalence of cretinism

The frequency of cretins occur in a targeted area, has been proposed as a measure of the presence and severity of IDD. This might serve admirably if the rate is sufficiently high and statistically reliable numbers can be obtained in reasonable time, and if the observers are sufficiently skilled in recognizing cretins apart from others with physical findings that superficially resemble those of cretinism. The clinical findings of cretinism are variable. The spastic paralysis, which tends to spare the distal ends of the extremities and other subtle and unique features of the endemic cretin may not be easily identified without experience and careful instruction. If endemic cretin constitutes one percent of the population then there has clearly been an IDD problem, but this is no quartette that the population is still at risk.

Dietary habits may have changed. It is always necessary to search for evidence of an existing or recent state of iodine deficiency.

4. Measurement of the severity of an IDD endemia

When an endemia of IDD has been identified it is necessary to know its severity in order that the urgency of intervention with a prophylaxis program can be judged. If the endemia is severe then there is great urgency to prevent the further devastating occurrence of cretins or neurologically damaged individuals in the community. This might call for an urgent and immediate program with iodize oil, because this can begin almost immediately, and further mental and physical retardation can be stopped at once. Generally programs of prevention with iodized salt take months or longer to implement because of the need to obtain the currency of governments and of the salt industry, to obtain the financing and the equipment for iodizing the salt, to develop a distribution and marketing system, and to promote the used of iodize salt by the general public. Guideline for classifying endemic appears in table. Should there be any question concerning where a given endemic fits, then the decision regarding prophylaxis should incline to greater concern and appropriate action, rather than the reverse.

Table 2. Summary of IDD prevalence indicators and criteria for a Public Health Problem.

Indicator	Severity of Public Health Problem		
	mild	moderate	severe
Goiter Grade > 0 %	5.0-19.9	20.0-29.9	≥ 30.0
Thyroid volume>97% percentile by ultrasound	5.0-19.9	20.0-29.9	≥ 30.0
Median urinary iodine level (μg/dl)	50.0-99.0	20.0-49.0	< 20.0
TSH > 10mU/L in serum	3.0-19.9	20.0-39.9	≥ 40.0

Background and rationale

IDD has long been public health problem of Thailand especially in mountainous area in the North of Thailand. WHO revealed in 1956 that IDD prevalence rate of two Northern province was 58% and 15-21% in North-Eastern. Later in 1957 WHO conducted resurveyed found goiter rate in five provinces of the North as high as 23.5-45.5%(6). For the eradication of endemic goiter, iodated salt was recommended as the prophylaxtic method of choice. Prof. Romsai Suwanik and his study group established the goiter control project in 1958 with the construction of a plant for the iodination of salt at Den Chai railway station, Phrae province in 1965. The high prevalence of goiter in Phrae (84.4%) was well controlled by iodated salt, reducing the incidence in schoolchildren to almost zero in 6 years (6). There are still some pockets of endemic goiter persist in the northern province because of irregular and inadequate iodized salt distribution. Since 1987, the national control program on IDD in the north of Thailand had been introduced. The program included epidemiologic survey and analysis, control methods for salt, water and fish sauce and practical exercises and management at the village level.

Mukdaharn Province is located in the North-easthern of Thailand, 642 kilometers from Bangkok. Prevalence of IDD was reported by Nutrition Division, Ministry of Public Health in 1989. The goiter rate was 30.5%. National IDD control program had been introduced by using iodating salt and water for a peroid of five years, after which the goiter rate was dropped to 12% by 1994. After that the oral iodized oil of 200 mgI was continued in Nong-ean-dong schoolchildren twice a year.

Two years after, the prevalence of goiter was increased to 22%. The program was discontinued for one year. In 1998, goiter prevalence was decreased to 16%. To control IDD in Mukdaharn Province, monitoring and evaluation was studied in Nong-ean-dong and Namteung schoolchildren, Khamcha-I district.

Objectives

1. To determine the severity of IDD in Nong-ean-dong and Namteung primary schoolchildren, Khamcha-I district, Mukdaharn province
2. To eliminate IDD in Nong-ean-dong schoolchildren, oral iodinating water of 150 μgI_2 / 100 ml /day was provided for a period of one year.
3. To monitoring and evaluation of IDD in Nong-ean-dong and Namteung schoolchildren, the volunteers were ascertaind as follow:
    1. Clinical evaluation of pre-and one year post-test;
        - Goiter prevalence by palpation
        - Thyroid volume by portable ultrasonography
        - The incidence of new cases of endemic cretin
    2. Biochemical evaluation
        - Urinary iodine excretion at 0,3,6,9 and 12 months
        - Thyroid function test of T_3, T_4, TSH, Tg, anti-Tg antibodies, and anti-microsomal antibodies at pre and one year post-test

CHAPTER II

LITERATURE REVIEW

In 1972, Thilly CH. et al. reported the existence of a very severe iodine deficiency in the population of Idjwi Island, with a goiter prevalence of 54% in one geographical region. However, the conclusion of this study is that the well-defined geographical distribution of endemic goiter on Idjwi does not correspond to any parallel variation in the iodine supply (17).

In 1975, Suwanik R. et al. described subjects of endemic goiter in Phrae and Nan provinces, showed low serum T_4, high serum T_3 and high ratio of T_3/T_4. The absence of hypothyroidism in endemic goiter is from the increase in efficiency of hormone synthesis and secretion as evidenced by elevated T_3/T_4. Thus the goiter is initially developed to compensate the iodine lack by increase in size, and by better quality of the work of the thyroid gland especially in the younger age group (18).

In 1976, Pleehachinda R. et al. assessed endemic and sporadic goiter in Phrae province, serum T_3:T_4 ratio was determined. It was found that the ratio in normal serum was less than 3. The higher ratio (>3) indicates iodine deficiency disorders (19).

In 1978, Tannahil AJ. et al. were calculating thyroid gland size from gray-scale ultrasound images in twenty patients with goiter. Results were compared with measurements by palpation and in some cases with measurements by scintiscan and at operation. There was a good correlation between ultrasound measurements and both

the sizes of surgical specimens and clinicians' estimations. Gland size calculated from scintiscan did not correspond well with measurements by ultrasound or palpation (20).

In 1981, Brunn J. et al. measured thyroid volume by real-time ultrasound in cadavers was compared with direct measurements obtained by submersion. Length x width x thickness of the thyroid lobe multiplied by factor pi/6, correspond to a rotation ellipsoid, while the best calculated volume of the lobe is obtained by multiplying with the optimized correction factor f = 0.479. The correctness of this calculation is, by definition, 100%; average error of the method is 16%. The measurements are easy to do and require no additional equipment for planimetry or calculations. Volumetric analysis of the thyroid gland is especially necessary in assessing results of treatment and for measuring dosage in connection with radioiodine therapy (21).

In 1982, Suwanik R. et al. Compared to the previous study (in 1963 and in 1969) of the serum findings of serum T_3 and T_4 still showed values within normal ranges after iodine replenishment, the urine iodine was definitely low whether a goiter was palpable or not in those schoolchildren. Thus mass screening by periodic measurements of urine iodine might be recommended for monitoring and evaluation of the iodine fortification program in Thailand (22). Pattanachak C. et al. found that, radioimmunoassay of T_3 and T_4 were modified by using 200 μg of 8-anilino-1-napthalene sulfonic acid for 1 ml of assay solution. The validation of the assays was shown by superimposable curves of standards in thyroxine free plasma in house, standard control (hypothyroid, hyperthyroid and normal serum) and standard serum from standard hormone. Precision and reproducibility of the assays were absolutely not significant ($p>0.05$). Concentrations of T_3 and T_4 by hand and standard hormone

kits were also very close and not significantly different ($p>0.05$) (23). Suwanik R. et al. studied the successful control control of goiter in nearby Phrae in 1968, the bottle neck is so present that palpable goiter is found in more than 80 per cent of the schoolchildren and presence of 4.4 % and 4.2 % of nervous type of endemic cretins in villages in Chieng Klang and Pua districts, Nan province. Even in Phrae's Wang Poong village, they have found 47 per cent of palpable goiter in schoolchildren and 8.9 & 28.9 per cent showed urinary iodine less than 50 & 100 μg/g of creatinine respectively (24).

In 1983, Suwanik R. et al. reported all of parameters, urine iodine excretion may be recommended to monitor the iodation programme to watch for re-apperance of goiter. Serum findings of T_4, T_3/T_4 and TSH measured different aspect, i.e. the extent of thyroid function to have resumed normal level. Recent studies in hyperendemic goiter area in Nan, originating from re-appearance of goiter in Phrae, showed that while findings in serum were near normal, the amount of iodine excreted via urine was much less than 20 μg/g creatinine among the grade Ia and Ib goiter of schoolchildren. Serum findings were not as sensitive a warning sign of goiter re-apprance as was urine iodine excretion (25). Suwanik R et al. studied the iodinating water was produced in particular on site by a small water supply from the filtering unit or water reservior used for drinking only. The quality of water at this low concentration of iodine is acceptable as it has no smell and no charge in test. However, trained volunteers should perform the monitoring on site. To prevent Jod Basedow disease the concentration of iodine should not exceed the level of 0.4 ppm and can be adjusted by valve when the monitor reveals developed colour to bee too dark (26). Bachrach LK. et al. studied

in 55 patient's age 6 days to 19 years for evaluating childhood thyroid disorders by ultrasonography. Findings were correlated with the available clinical, radionuclide, and pathologic data. They used 4 MHz internally focused at 4.5 cm with diameter 12.6-mm transducer. The method was based on recording crossection through the thyroid at 0.5 cm intervals, and a computer program employing semiautomatic digitizing of the outline the thyroid gland and calculated its. In addition, they found that thyroid ultrasound was a sensitive, noninvasive means of evaluating thyroid anatomy (27). Hegedus L. et al. measured the volume of the thyroid gland in vivo in 271 healthy subjects (13-91- yr.- old). The mean (± SD) thyroid volume was 18.6 ± 4.5 ml. A significant difference between males (19.6 ± 4.7ml; n = 139) and females (17.5 ± 4.2 ml; n = 132) was found ($p < 0.001$). The thyroid volume was significantly correlated with both body weight and ages, described by: $Y = 1.97 + 0.21\ x_1 + 0.06\ x_2$, where Y is the thyroid volume (milliliters), x_1 is the body weight (kilogrames), and x_2 is the age (years) (28).

In 1984, Pleehachinda R. et al. reported endemic goiter in the north of Thailand seems to be no problem since the introduction of iodated salt in the control of goiter 1963. Goiter still persists in some of those areas previously well controlled perhaps due to maldistribution of the iodated salt. In this communication, they report the persistence of goiter in the goiter villages and their schools in Phrae and Nan. They discussed the laboratory findings including the best ways to monitor a return of goiter and the incidence of mental deficiency in goitrous persons. The preventive measures might be extended to iodine water and other methods to ensure complete eradication of goiter (29).

In 1985, Bourdoux P. et al. concluded daily creatinine excretion is likely to depend on the protein intake , is not represent a satisfactory approximation to the daily excretion of iodine (16). Witherspoon LR. et al. used of magnetic-particle solid phase to estimation of T_3, T_4, TSH, FT_4 and T_3 uptake in 301 serum samples. They found that, the magnetic separations are rapid (2 to 5 minutes), noncentrifugation and excellent analytical performance (30).

In 1986, Gutekunst R. et al. described thyroid volume of 1,397 German and Swedish adults by ultrasound. Thyroid size of 6-16- yr.-old Germans (n = 619) was determined and compared with findings on palpation. Thyroid volume was great in German (21.4 ± 15.6 ml, mean ± SD) than in Swedish adults (10.1 ± 4.9 ml). German children had a thyroid volume ranging form 1.8 ± 0.4 ml at 6 years to 10.8 ± 0.4 ml at 16 years of age. In German, the iodine excretion was less in children (n = 619, 39.5 ± 30.5, 34.1 μg I/g creatinine, mean ± SD, median) than in adults (n = 1193, 83.7 ± 94.4, 62.6) ($P < 0.001$) and much lower than that observed in Swedish (adults = 98, 170.2 ± 93.3, 141.4; 13- yr.-old n = 113, 172.9, 224.1, 124) ($P< 0.0001$). Serum thyrotropin concentration was significantly higher ($p< 0.001$) in Sweden (n = 62.149 ± 0.82 mU/ml), than in Germany (n = 91, 0.97 ± 0.52 mU/ml), while serum thyroglobin was increased in Germany (n = 91, 72.6 ± 50.6 μg/l) as compared to Sweden (n = 62, 23.5 ± 17.4) ($P< 0.0001$) (31).

In 1988, Muller-leisse C. et al. were calculate thyroid gland from ultrasound data (using the ellipsoid formula) in 1080 apparently healthy schoolchildren, aged 7-20 years, from Spryer and Neckargemund in the Federal Republic of Germany. Thyroid volume was found to increase with age: mean of 4.34 ml (single standard deviation

(SD) 1.55) in 7-yr-old, 13.6 ml (SD 6.2) in 16-yr-old. No sex-related differences in size were found before the age ten years, with a slight size increase in girls at the time of puberty. The right lobe was significantly larger than the left one (mean difference of 0.8 ml) in all age groups. There was a weakly positive correlation for both sexes being between thyroid volume and age, body weight, height and surface area, respectively, the best correlation for both sexes being between eight with abnormal findings, all of them girls aged 16-18 yr-old (32). Tajtakova M. et al. examined thyroid volume by ultrasonography 40 years after prophylaxis of iodized salt. Thyroid volume was examine in 207 boys and 220 girls age 15-16 years from East Slovakia and it was found that 49.3% of the boys and 52.3% of the girls had thyroid between 10 and 15 ml. The findings were compared to other reports in 1949-53 before the introduction of iodine prophylaxis. They may be concluded that 40 years of goiter prophylaxis with iodize salt, though resulting in urinary excretion of approximately 100 μg/24 hr (as found by others in 1984), abolished large and medium size goiters in adolescents and decrease a prevalence of goiter grade I to about 25 percent (33).

In 1989, Ivarsson SA. et al. performed this study to determine the normal thyroid gland size in children of various ages living in Malmo, which was an iodine-sufficient area of Sweden. The material consisted of 66 healthy children whithout known thyroid disease, 29 boys and 37 girls form 5 to 16 years (median 10.5 years). The thyroid lobe was measured with a real- time ultrasound sector scanner (Diasonics DS 100) using a 10 MHz transducer. There was a continuous rise in thyroid gland volume form the age of 5 years to 15-16 years. No significant difference in thyroid volume between boys and girls. The thyroid gland volume in 5- to 6 -year-olds

children varied from 1.0 to 1.7 ml (mean volume 1.3 ml). In the 15-to 16-year-olds the thyroid varied from 4.1 to 10.1 ml (mean volume 7.0 ml). There was a significant positive correlation between the thyroid volume and the body area, weight, lenght and age ($r = 0.71$, 0.75, 0.78 and 0.82, respectively, $p < 0.00001$ for all). The regression line between the thyroid volume and body weight was $y = 0.130x - 1.286$ and between the thyroid volume and body $y = 6.87x - 3.881$ (34).

In 1990, Delange F. was reports an insufficient dietary supply of iodine of the fetus and young infants. The most serious consequence of iodine deficiency is the impact on neuro-intellectual, mental retardation to the complete picture of endemic cretinism. Considering that mental retardation due to iodine deficiency represents the long-term consequence of hypothyroidism occurring during the perinatal period. However, large areas or even countries in Europe are still obviously iodine deficient. For example, the iodine intake in adults in Belgium is 50 to 70 μg/day, which is lower than the recommended dietary allowance for iodine (at least 100 micrograms/day) (35). Hegedus L. was study the present that the ultrasonic evaluation of thyroid volume was both accurate and precise. The relationship between thyroid volume, body weight and age in non-goitrous healthy subjects can be described using a formula that allows the calculation of normal thyroid size for a population: Thyroid volume (ml) = 1.97 + 0.21 x bodyweight (kg) + 0.06 x age (years) (36). Tajtakova M. et al. studied the thyroid volume of ultrasound in a total of 921 boys and girls 6-16 years old. The thyroid volume was found to be increasing slowly between the age of 6 and 12 years, but somewhat more remarkable increase occurred at 13 and 14 years of age. In both sexes it was nearly doubled at the age of 15-16 years as compared with the

values at 13-14 years irrespective of body weight. The thyroid growth rate (as calculated from the least squares analysis of correlation between thyroid volume and body weight) in girls was significantly higher ($p < 0.001$) than in boys. In spite of long-term mandatory iodine prophylaxis the average urinary excretion of iodine as estimated in 69 randomly selected subjects was 78.16 μg/g creatinine (geometrical mean) (37).

In 1991, Chanoine JP. et al. were determine the volume of thyroid gland by ultrasonography in 256 euthyroid subjects aged 0-20 years in Brussels, an area with borderline iodine intake (median urinary iodine: 6.8 μg/dl). The volume of each lobe was calculated separately using the formula of an ovoid (Depth x Length x Width x $\pi/6$). The total thyroid volume was obtained by summation of the volume of both lobes. In neonates, mean volume (SD) was 0.84 (0.38) ml and the distribution was asymmetric, skewed towards elevated values (median: 0.76 ml); the volume was best correlated with body surface area ($p < 0.01$). Thyroid volume significantly increased ($p < 0.001$) until the age of 8 without being influenced by sex and thereafter varied widely: it increased from 2.7 (0.8) ml in puberty subjects age 8-11 years to 11.6 (4.4) ml in late pubertal aged > 17 years. This increase was significantly correlated not only with chronogical age with pubertal stage and seemed to happen early with the onset of the first clinical signs of puberty. At all ages, the volume of the right lobe was slightly higher than the left lobe but the difference was not significant (38). Simescu M. et al. studied on children aged 6-16 years. UNICEF and WHO initiated in 30 countries for assessing urinary iodine through the spot urine sample method. The urine samples

were collected from lots of 100-200 children aged 6-16 years within each district. The results showed low iodine values, i.e., 2.4- 5.5 μg /dl urine in 12 areas and 6-12.1 μg /dl urine in the rest of the country (39).

In 1992, Szebeni A. et al. were report a new simple, and accurate ultrasionic method is elaborated for thyroid volume determination with high- resolution real- time equipment. Applying the corrected ellipsoid model and computer-aided numerical integration did volume calculations. Comparing direct measurements of a silicone rubber phantom with ultrasonography of a tissue-equivalent thyroid phantom tested the accuracy of the method (40). Kinalska I. et al. surveyed in May 1991 in the city of Bialystok comprised 308 children of age between 8 and 14 years and 116 young adults. In about 50% of the subjects studied (58.4 % of children, 38.5% of men and 58.4% of women) the presence of goiter accompanied by a low urinary iodine concentration (median 2.0 μg /ml) was found. A significant negative correlation between the thyroid size and urinary iodine concentration. Higher prevalence of goiter found as compared to the results previously obtained in the same area can be related on the one to the use of more precise methods and on the other to real worsening of the situation due to discontinuation of obligatory iodine prophylaxis in the country more than 10 years ago (41).

In 1993, Dunn JT. et al. found that, urinary iodine excretion was currently the most convenient laboratory marker of iodine deficiency. It was rapid, simple methods for assessment and monitoring. They describe two adaptations of the Sandell-Kolthoff reaction. In which urine was first digested with Chloric acid and iodine then determined from its catalytic reduction of ceric ammonium sulfate in the presence of

arsenious acid. Both methods use gentle digestion by chioric acid in a heating block. Method A detects iodine in a colorimeter. Method B by the indicator ferroin and a stopwatch, Results with 12 samples ranging from 1.8 to 19.0 μg /dl (0.14-1.48 mumol/l) differed from those in a reference laboratory by a mean of 9.1% for method A and 15.7% for method B. One technician can perform at least 150 tests per day at a total cost of less than $0.50 each. The speed, low cost, and simple instrumentation make these methods well suited to epidemiological assessment of iodine deficiency in developing countries (42).

In 1994, Langer P. et al. were estimate thyroid volume in 4,254 schoolchildren and aldolescents 6-18 years of age from 12 districts of Slovakia and urinary iodine (by dry alkaline ashing followed by spectophotometry) in 1,174 spot urine samples. No differences in thyroid volume between sexes were found up to the age of 14 years, however, thereafter, such volumes were considerably higher in boys. When comparing their cummulated data with those reported by others for a population with optimal iodine uptake, it was found:1. the medians for most of the examined groups were slightly higher, 2. the percentage of values, which were higher than 97 percentiles of normal population, was 3.01 for the age of 6-14 years, while that for the age of 15-18 years was 9.04. Only 35.9% of all values of urinary iodine were in the optimal range(i.e. 10-20 μd/dl), while 56.1 were less than 10 μg/dl and 15.9 % less than 5 μg/dl, the remaining 8.0 % over 20 μg/dl. In spite of long term prophylaxis (since 1949), the intake of iodine apparently is still not satisfactory, since a considerable amount of individuals appeared to be iodine deficient on the day of examination (43). Vitti P. et al. used ultrasound machine to measure thyroid volume

in children and compared with thyroid palpation for the assessment of the prevalence of goiter in an area of mild iodine deficiency. Schoolchildren,6-14 years old, were from control area (n = 2,693; urinary iodine excretion,110 μg/L) or from an area of mild iodine deficiency (IDA; n = 278; urinary iodine excretion,72 μg/l). Thyroid volume determined by ultrasound in control children increased with age ($r = 0.62$; $p< 0.0001$) and was significantly correlated with the heigth($r = 0.51$; $p< 0.0001$) and body weight ($r = 0.126$; $p< 0.001$). Both median and mean thyroid volumes were greater in IDA children than in controls. The prevalence of goiter determined by ultrasound was 68 of 268 children (25.3%) in IDA and 105 of 2693 children (3.9%) in the control area ($\chi^2 = 204$; $p < 0.0001$). The thyroid enlargement, as assessed by palpation, was found in 59 0f 268 children (22%) in the IDA group and in 165 of 2693 (6.1%) subjects in the control area ($\chi^2 = 88$; $p< 0.0001$). Some subjects of the IDA who were judged goitruos by palpation (11.2%) had a normal thyroid volume at ultrasound, and 12.7% of subjects with an abnormal thyroid volume at ultrasound were judged nongoitrous by palpation (44). Delange F. reviewed present knowledge on the etiology, patho-physiology, complications, prevention, and therapy of the disorders induced by iodine deficiency. The recommended dietary allowances of iodine are 100 μg/day for adults and adolescents, 60-100 μg /day for children aged 1 to 10 years, and 35-40 μg/day in infants aged less than 1 year. The most important target groups to the effects of iodine deficiency from a public health point of view are pregnant mothers, fetuses, neonates, and young infants because the main complication of IDD, i.e., brain damage resulting in irreversible mental retardation, is the consequence of thyroid failure occuring during pregnancy, fetal, and early postnatal life. The most serious

complication of iodine deficiency is endemic cretinism, a syndrome characterized by irreversible mental retardation together with either a predominant neurological syndrome or predominant hypothyroidism, or a combination of both syndromes (45).

In 1995, Ares S. et al. were determine the volume of the thyroid gland by ultrasonography in 30 pre-term infants (27-36 weeks' gestation) born in Madrid. They found that thyroid gland volume significantly increased ($p< 0.01$) with postnatal and postmentrual age and was very well correlated with body weight, height and surface area ($p< 0.01$). Serum thyroid hormones T_3 and FT_4 were linearly correlated with postnatal and postmenstrual age, thus T_3 and FT_4 levels were also correlated with thyroid gland volume ($p< 0.05$). In addition, they found that quantitative determination of thyroid gland volume was more accurate for the diagnosis of goiter than clinical criteria (46). Calaciura F. et al. reported a case-control study of intellectual development, auxometric parameters and thyroid function performed in late infancy in children with documented transient congenital hypothyroidism or hyperthyrotrophinaemia at birth. Nine children born in an endemic goiter area who had short-term transient congenital hypothyroidism or hyperthyrotrophinaemia after birth (TCH) were studied and compared to nine matched children born in the same area at the same time but having normal thyroid function at birth (N). Global, verbal and performance IQs were evaluated on the Wechsler scale. Height, bone age, total and free thyroid hormones, thyroid volume, thyroglobulin, basal and TRH stimulated TSH were also measured. They found that height and bone age were similar in the two groups. Thyroid function tests were also similar in the two groups except for basal and TRH stimulated serum TSH and serum Tg which were performance IQs were

systematicall lower in the TCH than in the N group (78.3 ± 11.1 Vs 90.9 ± 14.2, $P < 0.05$; 84.4 ± 15.4 va 96.2 ± 14.8, PNS; 75.0 ± 8.5 va 89.2 ± 12.5, $P < 0.01$ respectively) (47). Riehl G. et al. assessed the prevalence of thyroid enlargement by ultrasonic volume measurements. The ultrasonic examination was carried out of 1,336 patients in the iodine-deficient area of Aachen, Weat Germany, who did not suffer from thyroid disease at the time of study. The thyroid volume was age-dependent and varied from 13.3 ± 10.4 ml in patients < 21 years to 29.2 ± 24.3 ml in-patients > 70 years. The prevalence of thyroid enlargement ranged from 14.3 % in young people to 51.3% in the elderly. There was no difference in the volumes of the left and right thyroid lobe. The prevalence of thyroid enlargement was higher in females compared to males ($p < 0.05$). Retrosternal thyroid mass was detected in 25% of all patients > 70 years. Finally they concluded that there was evidence of a high prevalence of thyroid enlargement in iodine-deficient areas (48). Wahl R. et al. prepared the standard food for volunteers in the University Hospital of Tubingen, approximately 230 micrograms of iodine/day. It was generally assumed that equilibrium was established between iodine intake from food and urinary iodine excretion. In 27 healthy volunteers the daily uptake of iodine was determined and the urinary excretion of iodine within 24 hr was measured. Unexpectedly, only 16% to 18% of the alimentary iodine were excreted with the urine. Hence, the utilization of iodine from the food appears to be limited and therefore cannot prevent goiters in goiter areas. In addition, it was found that the correlation between urinary excretion of iodine and urinary excretion of creatinine is not a reliable parameter for the real daily excretion of iodine and iodine balance (49).

In 1996, Kusic Z. et al. reported 40 years after introduction of iodine prophylaxis in Croatia. A total of 2,856 schoolchildren of both sexes, aged 7-15 years, were included into the study. The prevalence of goiter in schoolchildren was assessed by palpation and in part by ultrasonography of the neck. At the same time urinary iodine excretion was measured and iodine content in salt samples was determined. The results have revealed the persistence of mild endemic goiter in inland parts of Croatia with the prevalence of 6-29% in the age group 7-11 years and those of 10-43% among the age group 12-15 years. The overall goiter prevalence in schoolchildren in Croatia fluctuates from 8 % to 35% (50). Caron P. et al. studied the 'Thyromobile project' to update information on iodine supply in school-children aged 6-14 years in Europe. It involved 4 places in France: Lorraine, Rhone-Alpes, Languedoc-Roussillon, Midi-Pyrenees. With a mobile unit, all children (n = 1,522) had a thyroid echography performed by the same trained investigator, and urinary iodine concentration was determined in 1,458 of them. The mean ± SD of urinary iodine concentration was 13.0 ± 7.4 μg/dl, but 38% of urinary iodine concentrations were below 10 μg/dl and 10% were under 5 μg/dl. The prevalence of goiter was 4.1% in boys and 3.19% in girls. There was a negative correlation between urinary iodine concentration and thyroid volume ($P < 0.001$) (51). Pino S. et al. developed a safe alternative oxidizing reagent for measuring urinary iodine by using 1 mol/L ammonium persulfate. Further they found that this method is a nonexplosive, nonhazardous chemical (52).

In 1997, Liesenkotter KP. et al. monitored the iodine supply and its effect on the thyroid gland volume in prepubertal and pubertal children in the eastern and western parts of the city of Berlin. The thyroid gland volume was determined by

ultrasound in 1,080 (f = 552 , m = 528) children aged 3-15 years, and was correlated to age. Body-surface area and iodine excretion, which was measured in a first-morning spot urine. The mean iodine concentration was 115.8 µgI/g creatinine (12.2 micrograms iodine/dl urine) vs 116.7 µgI/g creatinine (12.0 micrograms iodine/dl) in the western parts of the city. The volume was found to increase with age and was 2.4 ± 1.1 ml in prepubertal (Prader and Largo: f < or = 10.9 ys, m < or = 11.5 ys) children, compared to 4.3 ± 1.7 ml in pubertal children (53). Pandav CS. et al. were studies of a cross- sectional study among class VI students studying in government schools of Delhi. A sample size of 1,200 was decided based on an expected prevalence of 50% with 5 % error and design effect of three. A trained doctor for the presence of goiter clinically examined all children in class VI of each school and casual urine samples were collected in capped plastic tubes. The urinary iodine estimation was done by the wet ashing method (54). WHO conducted among schoolchildren aged 6-15 years in 12 European countries provides ultrasound data for determining thyroid volume from 7,599 subjects, and urinary iodine levels from 5,709 subjects. A subgroup of 3,474 children born and living in areas where iodine intake is normal - as evidenced by median urinary iodine above 100 µg/l - furnishes data from which to derive thyroid volume reference values. This article presents the upper normal limit for thyroid volume, according to age, for the iodine-replete boys and girls in this subgroup, assessed using ultrasonography. Recommended upper normal limits of thyroid volume, calculated according to body surface area, are also reported. These cut-of values are recommended for interpreting survey and surveillance ultrasonography data among school-age children (55). Delange F. et al. were measure thyroid volume by

ultrasonography in 7,599 schoolchildren aged 7-15 years in one to fifteen site in The Netherlands, Belgium, Luxemburg, France, Germany, Austria, Italy, Poland,The Czechand Slovak Republices, Hungary and Romania.. The concentrations of urinary iodine were measured in 5,709 of them. A mobile unit (ThyroMobile van) equipped with a sonographic device and facilities for the collection of urine samples visited all sites in the 12 countries. It remains unchanged in other countries such as Belgium. There is an inverse relationship between urinary iodine and thyroid volume in schoolchildren in Europe. Goiter occurs as soon as the urinary iodine is below a critical threshold of 10 μg/dl. Its prevalence is up to 10-40% in some remote European areas. This work produced updated recommendations for the normal volume of thyroid measured by ultrasonography as a function of age, sex and body surface area in iodine-replete schoolchildren in Europe (56). Gartner et al. compared the iodine excretion and thyroid volumes in young students with or without continuous use of iodize salt in household. Students from five different universities in Bavaria were evaluated on a voluntary basis. Exclusion criteria were age above 30 years, known thyroid illness except simple goiter, application of x-ray contrast medium within the last 6 months or other known exposure to high doses of iodine. The participants answered a questionnaire indicating permanent ultilization of iodized salt for more than one year during all their meals, the intake of iodide tablets and in student cafeterias, offering meals that are prepared with iodized salt. Morning spot urine was drawn from each participant to measure creatinine correlated iodine excretion and thyroid volume was measured by ultrasound. In this study, 932 students could, mean age was 24 years, 501 female, 431 males. The mean iodide excretion in chronic

iodized salt user was 72.0 (± 68.7) μg/g creatinine compared to non-user 66.0 (± 65.4) μg/g creatinine ($p < 0.003$).Those students who were on iodide tablets had mean iodide excretion of 130.0 ± 720. μg/g creatinine. Mean thyroid volume was 14.1 (4.4 - 44.1) ml in females and 17.1 (6.6 - 64.4) ml in males (57). Aghini-lombardi F. et al. evaluated prevalence of goiter by ultrasound in the schoolchildren population of an area of EasternTuscany (Tiberina Valley) characterized by moderate iodine deficiency in 1985. At present, after the implantation of voluntary iodized salt consumption, iodine urinary excretion was boderline sufficient (median,98 μg/L). Goiter prevalence was higher at ultrasound (17%) than by palpation (10%). The median thyroid volume range from 3.1 ml in 7 -yr-old children to 9.2 ml in 14 -yr-old children. In the 7-10 yr age class (i.e. in children born after iodine prophylaxis), no statistical difference in thyroid volume was found with respect to controls. In older children (11-14 yr.) born before the institution of iodine prophylaxis, the median thyroid volume was significantly higher than that in age-matched controls. Moreover, in this cluster of subjects, the median thyroid volume in nongoitrous children was higher than controls (58).

CHAPTER III

MATERIALS AND METHODS

Materials

1. Instruments

Table 3. Instruments were provided in the studies.

No	Name	supplier
1.	Automatic gamma counter	Wallac, 1470-010 Wizard, Findland
2.	Refrigerated centifuge machine	Beckman J6- MI , USA
3.	Spectrophotometer	Milton Roy Spectronic 1001 plus, USA
4.	Heating block	Fisher Scientific, USA
5.	Fume hood for perchloric acid	Laboconco Corporation, USA
6.	Milli-Q Plus water system	ZD 52 11584, USA
7.	Deminalizer	Ablauf , Germany
8.	PH meter	Orion Research Microprocessor Ionalyzer /901, USA
9.	Vortex mixer	Vertex-2 Genie, Scientific Indrustries, USA
10.	Analytical balance	Mettler H 31 AR, Switzerland. Mettler P1210, Switzerland
11.	Hot plate with stirrer	Monotherm, Germany
12.	Laboratory thermometer	
13.	Portable ultrasound scanner with 7.5 MHz transducer (Linear)	ALOKA SSD 500, Japan
14.	Repeated automatic pipettes ; 25, 50, 100, 500 µl	Eppendorf, Germany
15.	Polystyrene tubes : size10 x 65 mm	Elkry, USA
16.	Glass tubes : size 13 x 100 mm	Pyrex, USA
17.	Test tube rack	
18.	Microtritre plate 96 wells	
19.	Rotator	LEEC Limited, UK

2. Chemicals.

Table 4. Chemicals were provided in the studies.

No	Name	supplier
1.	Disodium hydrogen phosphate (Na_2HPO_4)	E. Merk, Germany
2.	8-Anilino-1-napthalene sulfonic acid (ANS)	Sigma, USA
3.	Sodium dihydrogen phosphate	E. Merk, Germany
4.	Polyethyleneglycol (PEG 6000)	E. Merk, Germany
5.	Standard L-T_3 and L-T_4 acid free	Sigma, USA
6.	Sheep anti-T_4 antiserum	NETRIA, UK
7.	Bovine serum albumine (BSA)	Sigma, USA
8.	Donkey anti-sheep serum (DASS)	NETRIA, UK
9.	Hormone free serum (HFS)	Inhouse
10.	Labelled triiodothyronine (^{125}I- T_3)	NEN, USA
11.	Labelled tetraiodothyronine (^{125}I- T_4)	NEN, USA
12.	Q.C serum: low, medium, high for T_3, T_4, and TSH	Inhouse
13.	Thyroglobulin comercial kits (ELSA-hTG)	CIS biointernational,France
14.	Standard TSH	Office of Atomic Energy for Peace(OAEP)
15.	Labelled monoclonal anti- TSH (^{125} I-mAb)	OAEP
16.	Anti-TSH solid phase (Anti-TSH-CDI-cellulose)	OAEP
17.	Magnetic particle (MP) anti – T_3 antibodies	Inhouse
18.	Thymune-M and Thymune-T kits	Murex, UK
19.	Potassium chlorate($KClO_3$),dry powder	Merck 4944,Germany
20.	Perchloric acid ($HClO_4$) 70%	Merck 159,Germany
21.	Arsenic trioxide (As_2O_3), dry powder	Merck 119,Germany
22.	Sodium chloride(NaCl), dry powder	Merck 6404,Germany
23.	Sulfuric acid (H_2SO_4), 95-97% sp.gr.1.84	Merck 731,Germany
24.	Ceric ammonium sulfate ($Ce(NH_4)_4(SO_4)_4.2HO_2$), dry powder	Merck 2273,Germany
25.	Potassium iodate(KIO_3), dry powder	Merck 505,Germany
26.	Nitric acid (HNO_3) 65%	Merck 456,Germany
27.	Deionized water	
28.	Ultrasonic gel	

3. Reagents

A. Radioimmunoassay of serum T_3

1. Assay buffer: 0.05 M Veronal buffer PH 8.6 with 2% Triton-x , 5% BSA
2. Washing buffer : 0.05 M Phosphate buffer PH 7.4
3. Anti-T_3-antibody magnetic cellulose
4. Uncoupling magnetic particle for NSB
5. Labelled triiodothyronine (^{125}I-T_3)
6. Control serum of low (40 ng/dl), medium (120 ng/dl) and high (250 ng/dl)
7. Standard serum of 0, 50, 100, 150, 200, 300, 500, 800 ng/dl

B. Radioimmunoassay of serum T_4

1. Assay buffer : 0.05 M Phosphate buffer PH 7.4 with 10 % Sodium azide
2. 4 % PEG 6000 with 10 % Sodium azide, 10 % Triton-x100
3. HFS
4. Sheep anti-T_4 antiserum
5. Donkey-anti-sheep serum (second antibody)
6. ^{125}I-T_4 with ANS 4 mg/ml
7. Control serum of low (2 µg/dl), medium (6 µg/dl) and high (12 µg/dl)
8. Standard serum of 0, 1.25, 5.0, 7.5, 10, 15, 20 µg/dl

C. Immunoradiometric assay of serum TSH

1. Assay buffer : 0.05 M Phosphate buffer with 0.1 % Sodium azide, 0.05 % Tween-20 and 1 % BSA
2. Labelled monoclonal anti-TSH or ^{125}I-mAb
3. Anti-TSH-solid phase
4. Washing buffer : 0.05 M Phosphate buffer PH 7.4 with 0.1 % Sodium azide, 0.05 % Tween-20
5. Standard serum of 0, 0.2, 1.5, 3.0, 10, 30, 60 mIU/L for TSH
6. Control serum of low (1.0 mIU/L), medium (20.0 mIU/L) and high (33.0 mIU/L)

D. Urinary iodine excretion

1. Chloric acid solution ($HClO_3$) : 500 gm of $KClO_3$ in 910 ml of deionized water with 375 ml of $HClO_4$ (70%)
2. Arsenious acid solution : 0.5 % As_2O_3 with 2.5 % NaCl in 400 ml of 5 N H_2SO_4 and adjusted volume to 2 L with deionized water
3. Ceric ammonium sulfate solution : 2.4% Ceric ammonium sulfate in 1 L of 3.5 N H_2SO_4
4. Potassium iodate standard (KIO_3) : Stock standard iodine 100 µg/ml and working standard iodine 0.5 µg/ml
5. Control urine iodine of low (25-35 µg/L), medium (35-55 µg/L) and high (95-100µg/L)

Methods

The 624 healthy schoolchildren aged 8-11 years without known thyroid disease in Bangkok were studied for normal thyroid size by palpation, ultrasonography and urinary iodine excretion.

543 schoolchildren of both sexes and aged 6-14 years in Namteung subdistrict, Khamcha-I district, Mukdaharn province were studied for prevalence of IDD. The volunteers were devided into 2 groups, placebo in Namteung (n = 338) and treated in Nong-ean-dong school (n = 205). Iodating water of 150 μgI/day and water without iodine fortifications (median iodine content was 25.43 μg/L) were given to those in treated and placebo at lunch time for one year respectively. Measurement the presence of IDD and follow up after prophylaxis in both groups were performed as follow:

Estimation of thyroid volume size by palpation

The thyroid size were measured according to present criteria of WHO/UNICEF/ICCIDD in 1992, which is described in Table 1

Thyroid volume by ultrasonography

A portable ultrasonic scanner with 7.5 –megahertz linear transducer was used to determine the thyroid volume. Parasagittal and transverse scans were done at the anatomical site of thyroid gland with hyperextension of the neck allowing the measurement of length , width and thickness of right and left lobes as shown in Fig 4 and Fig 5. Thyroid volume was calculated by Brunn's formula : volume of one lobe = (0.479)(length)(width)(thickness). The total volume was obtained by addition of the volume of both lobes. Body surface area was also calculated according the formula BSA = weight $(kg)^{0.425}$ x height $(cm)^{0.725}$ x 71.84×10^{-4} (59).

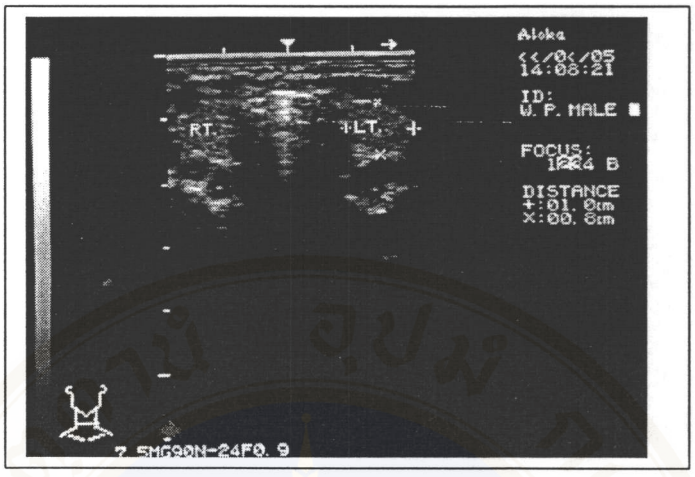


Fig. 4. Ultrasonography : transversal section of left and right thyroid ultrasonography.

Fig. 5. Ultrasonography : parasagittal section of thyroid ultrasonography.

Urinary iodine excretion (60,61)

3 ml of casual urine samples of placebo (n = 89) and treated (n = 117) were collected in plastic tube and kept in refrigerator (4°C) or freezer (-20 °C) for longer stability. Urine iodine was analyzed by modified PAMM method. All of laboratory test tubes were marked at 1 ml so all solution can be diluted to 1 ml. Urine

sample was mixed to suspend sediment and pipette 250 µl of each sample into 13 x 100 mm test tube. Duplicate of standards were prepared by pipetting 0,10, 20, 40, 60 and 80 µl into test tubes and adding 250, 240, 230, 210, 190 and 170 µl deionized water to these tubes respectively to give a volume of 250 µl in each tube. This gives a standard curve ranging from 0, 20, 40, 40, 120 and 160 µg/L. 750 µl of chloric acid was added into each tube and mixed gently. All tubes were heated for 50 minutes at 117 °C in fume hood with a perchloric acid trap, and cooled tube to room temperature. Each tube was diluted to the 1 ml mark. 3.5 ml of arsenic acid solution was added into each tube, mixed and standed for about 15 minutes. 700 µl of ceric ammonium sulfate solution was added to each tube at 30 seconds time intervals, mixed each tube after the addition. Exactly thirty minutes after the addition of ceric ammonium sulfate to the first tube, read its absorbance at 405 nm, and read successive tube at 30 seconds time intervals. Constructed a standard curve by plotting log (absorbance at 405 nm) versus the standard iodine concentration. For each sample, found its log (absorbance) on the standard curve and read off the corresponding iodine concentration (µg/L).

In-house radioimmunoassay of triiodothyronine employing magnetic particles method (62, 63)

Laboratory test tubes were prepared and labelled in duplicate tubes for total count (TC); non specific binding (NSB); standard T_3 of 0, 50, 100, 150, 200, 300, 500, 800 ng/dl; control serum T_3 of low, medium, high; and serum samples. 50 µl of HFS was pipetted into NSB and standard of 0.50 µl of standards, controls, and samples were pipetted into standard tubes; control low, medium, high tubes; and sample tubes. It was important to pipette 50 µl of ^{125}I -T_3 into each tube and mix the

solution by vortex mixer. 50 µl of anti-T_3-magnetic cellulose were added into each tubes (except NSB tubes, added 50 µl of uncoupling magnetic cellulose for NSB), and 50 µl of T_3 buffer was added. It was necessary to mix the solution by vortex mixer. The assay solution tubes were rotated on rotator for 4 hours at room temperature. 1 ml of washing buffer were added twice into each tube and the supernatant was decanted by magnetic rack. The bound fraction was counted for 1 minute by automatic gamma counter. A standard curve was constructed by microcomputer (plotting cpm versus standard concentration). Finally, the corresponding T_3 concentrations (ng/dl) of each sample was read on the standard curve.

Radioimmunoassay of thyroxine (T_4) by In-house double antibody PEG (64).

Laboratory test tubes were prepared and labelled in duplicate tubes for TC; NSB; standard T_4 of 0, 1.25, 2.5, 5, 7.5, 10, 15, 20 µg/dl; control serum T_4 of low, medium, high; and of unknown serum samples. 25 µl of the standards, serum samples were pipetted into standard and sample tubes. 25 µl of HFS was pipetted into standard T_4 of 0 and NSB tubes. It was significant to add 50 µl of 20,000 count per minute of ^{125}I-T_4 with 4 mg/ml of ANS . Added 50 µl of anti-T_4 into each tube (except NSB tube). 50 µl of DASS was added into each tube (except TC tube), and 100 µl of assay phosphate buffer 0.05 M was added into each tube (except NSB tube added 150 µl). It is necessary to mix the solution by vortex mixer and incubate at room temperature (23-25 °C) for 2 hours. 500 µl of 4% PEG was pipetted and mixed. The precipitation was collected by centrifugation at 3,000 rpm for 30 minutes. The

supernatant was discarded in 15 minutes. The precipitate was counted in automatic gamma counter for 1 min. A Standard curve was constructed by using RIA-Cal-program (plotting B/T versus the standard T_4 concentration). Finally, the corresponding T_4 concentration (μg/dl) of each sample was read on standard curve.

In-house immunoradiometric assay (IRMA) of TSH in serum using monoclonal anti-TSH (65).

It was necessary to prepare and label laboratory test tubes in duplicate tubes for total ^{125}I-anti TSH count (TC); standard TSH of 0, 0.25, 0.5, 1.5, 3.0, 7.5, 30, 60 mU/L; control serum TSH of low, medium, high; and serum samples. 100 μl of the standards and serum samples were pipetted into standard and sample tubes and then 300 μl of assay buffer was pipetted into each tube (except TC tubes). 50 μl of ^{125}I-anti-TSH was pipetted into each tube and 50 μl of anti-TSH solid phase was added during mixing (except TC tubes). The final mixture tubes were mixed by vortex mixer and rotated on rotator at room temperature over night. The solid phase was washed twice by adding 1 ml of wash buffer, mixed on vortex mixer, precipitated in refrigerated centrifuge, and decanted the supernatant. The radioactivity of precipitate in each tube was counted for 150 seconds by automatic gamma counter. A standard curve was constructed by RIA -Cal –program (plotting cpm versus the standard TSH concentration). Finally the corresponding T.S.H. concentration (mIU/ L) of each sample was read on the standard curve.

Immunoradiometric assay of serum thyroglobulin (Tg) by commercial ELSA-hTG kits

The kits were stored at 2-8 °C until the expiry date. All of reagents were also brought to room temperature (18-22 °C) at least 30 minutes before their used. The ready for used of monoclonal anti-Tg antibody coated on the ELSA fixed at the bottom of the 96 tubes was used for the assay tubes. The assay requires the following groups of tubes : 0, 1.5, 5, 15, 50, 150, 500 ng/ml of standard serum hTg, control serum hTg, test serum hTg, unknown serum and interference test. The assay was done in duplicate except interference test. The standard lyophilized serum Tg, control and test serum were diluted with distilled water as shown in the direction before used. The interference test was performed by diluted unknown serum sample with test serum Tg in the ratio of 1:1. 300 µl of buffer was added into all ELSA tubes. 100 µl of standard, control, test serum hTg, samples and interference test were pipetted into the corresponding groups of tubes. Mixed gently each ELSA tube with vortex mixer. Incubated for 3 hours ± 5 minutes at room temperature (18-25 °C) under agitation. The ELSA tubes were washed as follows: aspired in the content of the tubes as completely as possible. 3 ml of washing solution was added to each tube before emptying them again. The process was repeated once more. Ready for used 300 µl of 125I-anti-Tg monoclonal antibody was added each ELSA tube. Mixed gently each ELSA tube with vortex mixer. Incubated for 18-24 hours at room temperature (18-25°C). The ELSA tubes were washed again as previously described. The radioactivity bound to the ELSA tube was measured by automatic gamma counter. A standard

curve was constructed and the corresponding Tg concentration (ng/ml) of each sample was read on standard curve by RIA-Cal-program.

Measurement of anti-microsomal antibodies in serum by commercial MEREX kits

The patients' sera were heat inactivated at 56 °C for 30 minutes. Diluent of 0.1 and 0.075 ml was pipetted into microtitration plate of well 1-2 and well 3-12 respectively. 0.025ml of serum was pipetted into well 1. A micropipette or microdiluter was used for mixing and transferring of tip,0.025 ml of well 1 into well 2. With a clean micropipetted, 0.025 ml of solution in well 2 was transferred to well 3 (serum control) and well 4. A clean micropipette tip or microdiluter was also used for mixing in well 4 and transferring of 0.025 ml from well 4 to well 5, and continuing four folds dilution to well 12. 0.025 ml of solution in well 12 was discarded. The total solutions of well 1 to 12 were 1:15, 1:25, 1:100, 1:200, 1:400, 1:1,600, 1:6,400, 1:25,600,1:102,400,1:409,600, 1:1,638,400, 1:6,553,600 respectively. The solution of 0.025 ml of control and test cells were added immediately in to well 3 and well 4-12 respectively. Mixed contents on a plate shaker for a minimum of 30 seconds. Left the plate to settle at room temperature out of direct sunlight and free from any vibration. Read the liter after one hour.

Measurement of anti- thyroglobulin antibodies in serum by commercial MUREX kits

The patients' sera were heat inactivated at 56°C for 30 Minutes. 0.1 ml of diluent was pipetted into well 1 titration, and 0.025 ml into well 2-12 of microtitration plate. A clean micropipette or microdiluter was used for mixing in well 1 and transferring of 0.025 ml into well 2 (serum control well). 0.025 ml of solution in well 2 was discarded. A clean micropipette was used for mixing in well 1 and transferring of 0.025 ml into well 3, mixed and transfer of 0.025 ml into well 4. Continue the doubling dilution to well 12. 0.025 of solution in well 12 was discarded. Total dilution of well 1-12 were 1:5, 1:10, 1: 20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1,280, 1:2560, 1:5120 respectively. 0.025 of control cell was added into well 2 and 0.025 ml of test cell was added into well 3-12 immediately. Finally, a plate shaker was used to mix for a minimum of 30 seconds. Left the plate to settle at room temperature out of direct sunlight and free from vibration. Read the titre after 30-60 minutes.

Test result of Antimicrosomal and Antithyroglobulin

In a positive test the sensitized cells are agglutinated by antibody and settle to the bottle of the well as a diffuse carpet. In a negative test the cells settle as a small circle or compact button at the bottom of the well. Weakly positive reactions may result in intermediate patterns. The end point should be read as a highest dilution of the sample giving approximately 50 % agglutination of the test cells.

Statistical analysis was performed by the Statistic package for Social Sciences (SPSS 9.02).

CHAPTER IV

RESULTS

Pre-test of goiter palpation, thyroid volume by ultrasonograph, serum findings and urine iodine were performed in Khamcha-I schoolchildren as shown in table 5. Goiter prevalence of treated (20.00%) was higher than placebo (4.58%) 4.4 times. All parameters of serum findings, urine iodine and thyroid volume of schoolchildren aged 8, 9, 11 years were non-significant difference between treated and placebo group except thyroid volume aged 10 yrs ($P < 0.001$).

Table 5. Goiter prevalence, thyroid volume, serum findings and urinary iodine in pre-test of treated and placebo group.

Indicator	Mean ± SD (n)		
	Treated	Placebo	P-value
1. Goiter prevalence	20.00% (85)	4.58% (131)	
2. Thyroid volume (ml)			
8 yrs	3.48 ± 1.10 (17)	3.05 ± 0.59 (15)	0.190
9 yrs	3.98 ± 1.85 (26)	3.37 ± 0.98 (56)	0.053
10 yrs	5.32 ± 2.25 (36)	3.83 ±1.02 (48)	<0.001
11 yrs	6.39 ± 2.86 (6)	5.19 ±1.28 (12)	0.366
3. Serum			
Tg (ng/dl)	9.17 ± 6.85 (56)	9.90 ± 7.76 (21)	0.688
T3 (ng/dl)	131.20 ± 36.28 (147)	132.51 ± 36.67 (67)	0.818
T4 (μg/dl)	7.50 ± 1.61(147)	7.93 ± 1.51 (68)	0.069
TSH (mIU/L)	2.59 ± 1.36 (147)	2.64 ± 1.31 (68)	0.516
Anti-Tg antibody	Negative	Negative	
Anti- Microsomal antibody	Negative	Negative	
4. Urinary iodine (μg/L)	56.61 ± 21.55 (117)	62.34 ± 21.86 (87)	0.063

Thyroid volume reference value were also derived using data from schoolchildren (338 boys and 304 girls) living in normal iodine intake (median urinary iodine was 126.55 μg/L) as shown in table 6.

Table 6. Normal thyroid volume (ml) of schoolchildren aged 8-11 years in BKK.

Age (yrs)	n	Mean	SD	Range	Median	P 97
8	88	2.15	0.74	0.73 - 5.41	2.01	3.94
9	203	2.32	0.71	1.04 - 5.62	2.21	3.98
10	207	2.72	0.88	0.63 - 5.75	2.59	4.73
11	144	2.72	0.94	1.07 - 5.86	2.61	5.13

The thyroid volume in 8- to 9- year-old students varied from 0.73 to 5.62 ml and the mean volume was 2.15 and 2.32 ml respectively. In the 10 to 11-year-olds, the volume varied from 0.63 to 5.86 ml and the mean volume was 2.72 ml. There was significant difference between 8-to 9-and 10- to 11-year old students ($P < 0.05$).

The upper normal limit of thyroid volume (P 97) was 3.94, 3.98, 4.73 and 5.13 ml in the 8, 9, 10 and 11-year-olds respectively. There was a continuous increase in thyroid volume calculated by P 97 with age (Fig. 6). The mean and median were slightly decrease in aged 11 yrs.

No significant difference in thyroid volume between boys (n = 338) and girls (n = 304) aged 8-11 years was observed. The thyroid volume of left lobe was not significant difference from right lobe ($P > 0.05$, n = 642).

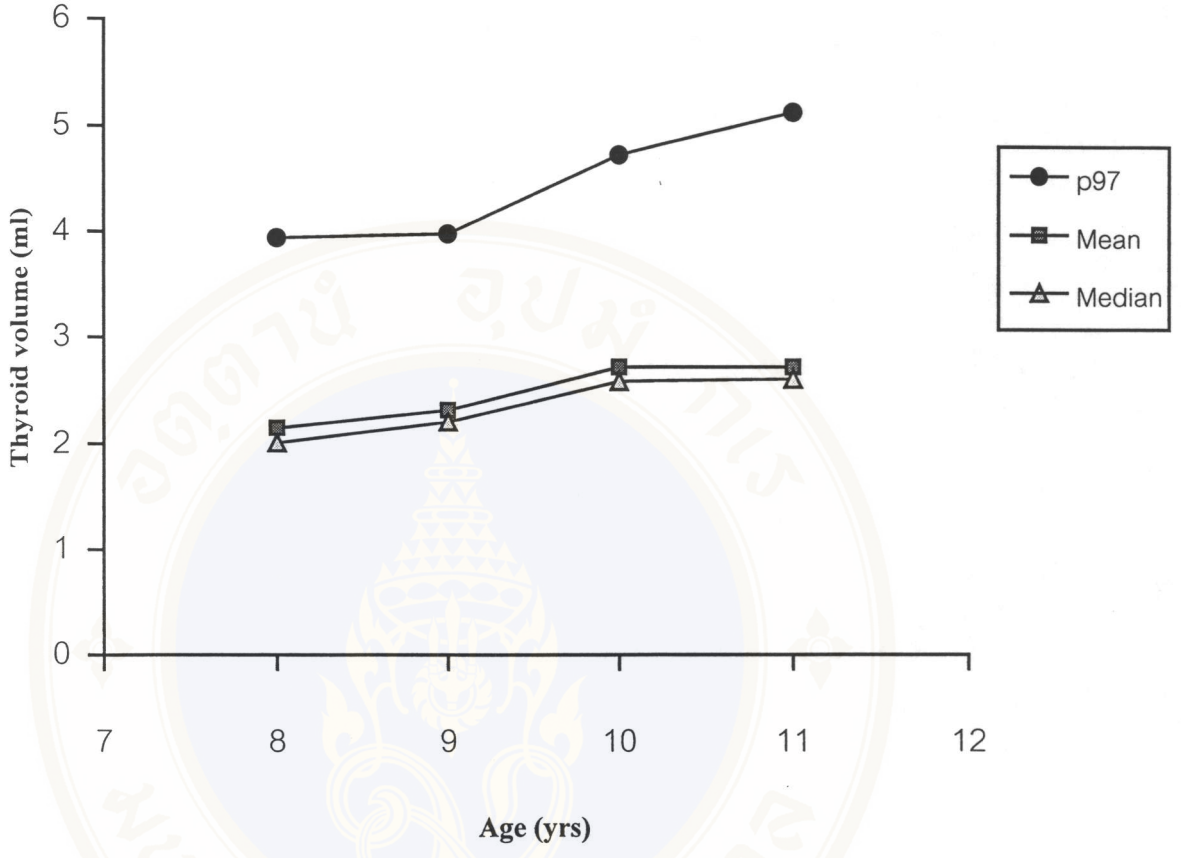


Fig. 6. Thyroid volume (ml) of schoolchildren in iodine sufficient area (Bangkok).

Due to the time consuming, the thyroid gland volume in normal schoolchildren aged 8, 9, 10 and 11 yrs was calculated by using transverse scan. The thyroid volume was obtained by multiplying width and thickness with the optimised correction factor (cf) of 1.099, 1.224, 1.455 and 1.376 respectively. There was no significant difference of thyroid volume in schoolchildren aged 8-11 years between Brunn's and our formula (Table 7).

Table 7. Thyroid volume of schoolchildren aged 8-11 year olds in BKK and Mukdaharn were calculated by Brunn's (I*) and our formula (II**).

Age	n	Mean ± SD Thyroid volume (ml) I*	Mean ± SD Thyroid volume (ml) II**	Correction factor (cf)***	P-value
8	93	2.280 ± 0.793	2.294 ± 0.716	1.099	0.519
9	226	2.542 ± 0.760	2.556 ± 0.717	1.224	0.343
10	221	3.027 ± 1.000	3.038 ± 0.883	1.455	0.531
11	134	2.837 ± 0.936	2.872 ± 0.819	1.376	0.075

* Thyroid volume = (width)(thickness)(length)(0.479)
**Thyroid volume = (width)(thickness)(cf)
cf*** = Mean of thyroid volume I* / Mean of (width)(thickness)

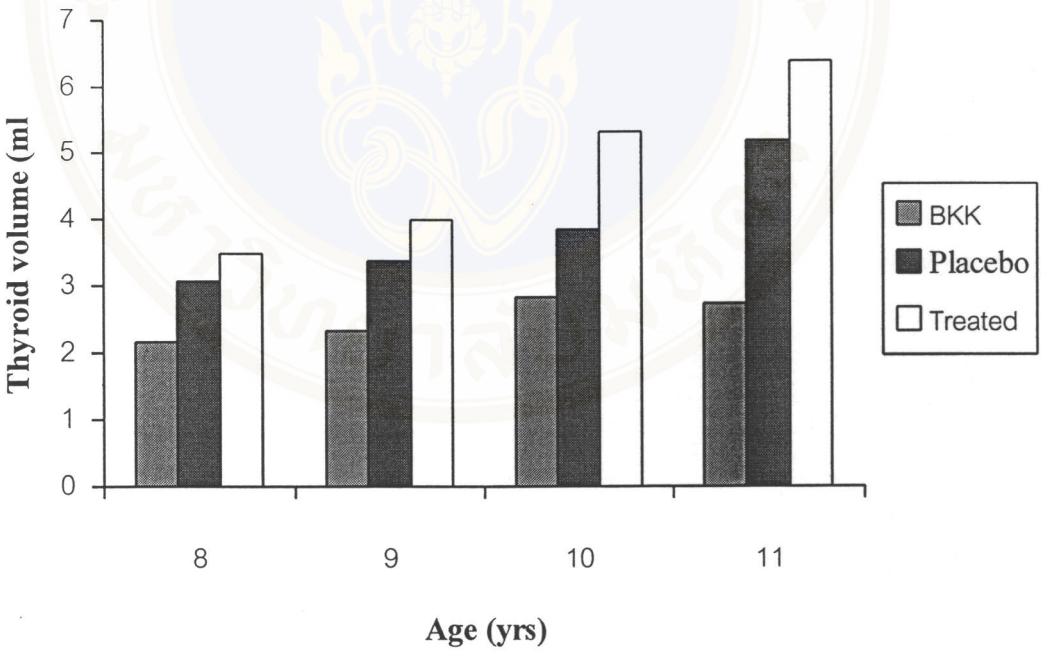


Fig. 7. Pre-test of mean thyroid volume (ml) in iodine-replete (BKK), iodine-deficient; treated and placebo schoolchildren aged 8-11 years.

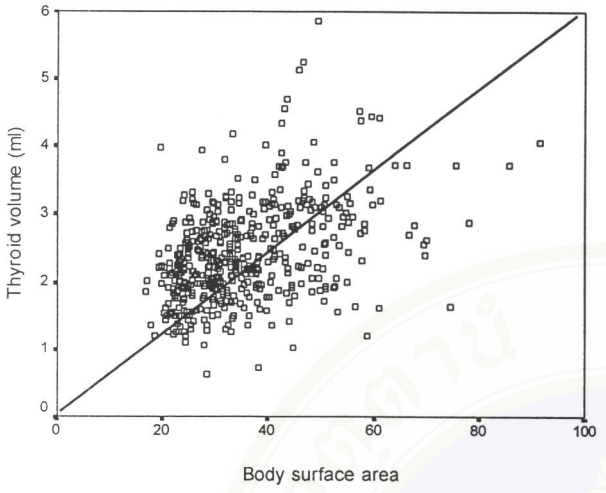
Fig 7. shows thyroid volume in pre-test of normal (Bangkok), and iodine deficiency area in Mukdaharn province: Namteung (placebo) and Nong-ean-dong (treated). The mean thyroid volume measured by ultrasonography in BKK; placebo; treated were 2.15, 2.32, 2.72 and 2.72 ml; 3.05, 3.37, 3.83 and 5.19 ml; 3.48, 3.98, 5.32 and 6.39 ml of schoolchildren aged 8, 9,10,11 years respectively (Table 5, 6).

Table 8. The relationship between thyroid volume and age, body weight, body height and body surface area in normal schoolchildren (BKK).

Parameter	n	r	P
Age	507	0.311	< 0.01
Body weight	492	0.389	< 0.01
Body height	492	0.440	< 0.01
Body surface area	492	0.432	< 0.01

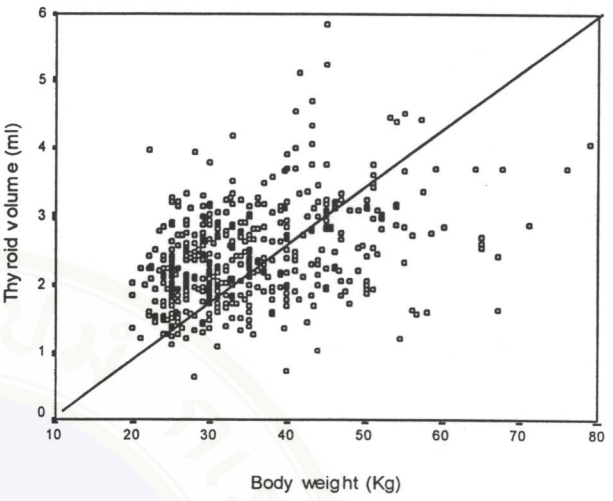
Table 8. shows significant positive correlation between the normal thyroid volume and the age, weight, height and body surface area ($p<0.01$ for all). The age correlates less to thyroid volume than the body weight, height and body surface area. The best correlation is found between body height and thyroid volume.

The regression line between the thyroid volume and body surface area was $y = 0.02527x + 1.526$, between the thyroid volume and body weight $y = 0.02757x + 1.449$, between thyroid volume and age $y = 0.227x + 0.254$, and between thyroid volume and body height $y = 0.042x - 3.334$ (Fig.8).



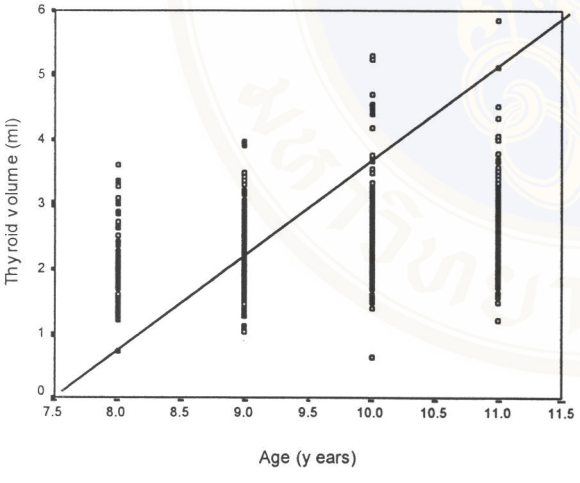
(a) y = 0.02527x + 1.526

n = 492, r = 0.432



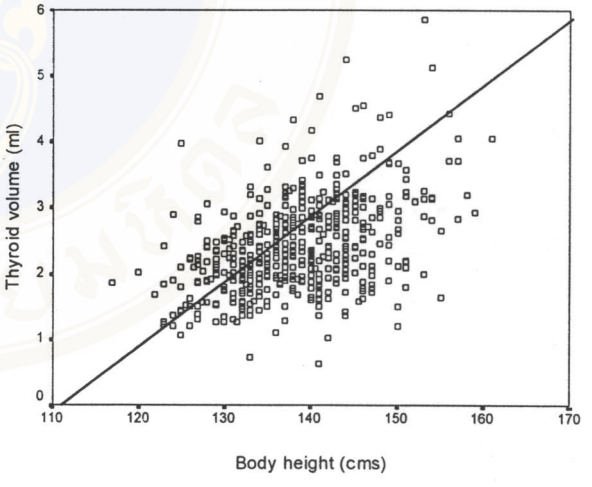
(b) y = 0.02757x + 1.449

n = 492, r = 0.389



(c) y = 0.227x + 0.254

n = 507, r = 0.311



(d) y = 0.042x – 3.334 (n = 492)

n = 492, r = 0.440

Fig. 8. Correlation between thyroid volume and body surface area (a), body weight (b), age (c) and body height (d).

Prevalence of IDD in Khamcha-I district is shown in Table 9.

Table 9. Summary of IDD Prevalence indicator and criteria for a Public Health Problem in pre-test of Khamcha-I schoolchildren.

Indicator	n	Percent	μgI/L	Severity of IDD
Treated				
1. Goiter grade > 0	85	20.00	-	Moderate
2. Thyroid volume by ultrasound (>P97)	85	44.71	-	Severe
3. TSH > 10 mIU/L serum	139	0.70	-	No
4. Median urinary iodine level	117	-	56.6	Mild
Placebo				
1. Goiter grade > 0	131	4.58	-	No
2. Thyroid volume by ultrasound (>P97)	131	19.08	-	Mild
3. TSH > 10 mIU/L serum	68	0	-	No
4. Median urinary iodine level	87	-	62.3	Mild

Table 10. %Goiter prevalence of pre-and one year post-test by palpation in Khamcha-I district.

Subjects	Pretest			Post-test		
	n	Goiter grade ≥1	% Prevalence	n	Goiter grade ≥1	%Prevalence
Placebo	131	6	4.58	113	6	5.31
Treated	85	17	20.00	84	11	13.09

Table 10. shows decreasing in prevalence of goiter in schoolchildren from 20.00 to 13.09 % after receiving iodating water 150 μgI/day for one year in treated group. In placebo group, the prevalence in post-test was 5.31 %, which is slightly higher than pre-test of 4.58% (Fig 9.).

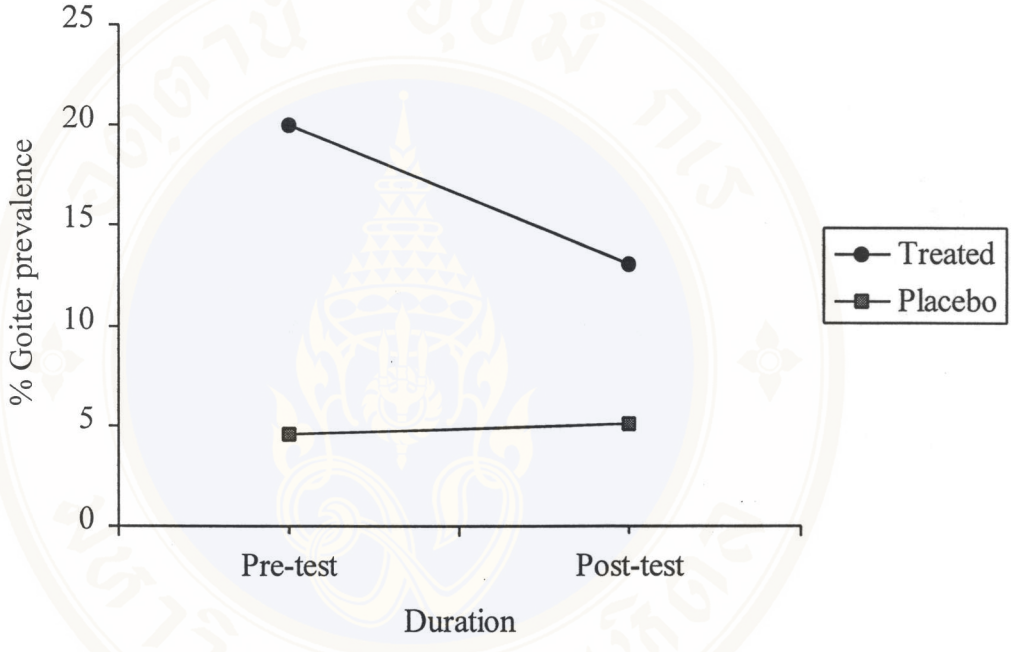


Fig. 9. Percent of goiter prevalence by palpation of placebo and treated in Khamcha-I district.

Urinary iodine excretion in treated group was increased from 59.1 to 180.6, 165.7, 127.6 and 171.8 μgI/L in 3, 6, 9 and 12 months respectively (Table 11. and Fig. 10). These urine iodine levels were higher than normal median value of 126.6 μg/L. In placebo, the level of urine iodine was also increased from 64.2 to 103.4, 107.5, 90.6 and 98.6 μg/L respectively but lower than the normal limit.

Table 11. Median urinary iodine excretion (μg/L) of schoolchildren in Khamcha-I district at 0, 3, 6, 9 and 12 months.

	Median Urinary iodine excretion (n)				
	Pretest	3 mo	6 mo	9 mo	12 mo
Normal	126.6 (611)	-	-	-	-
Placebo	64.2 (87)	103.4 (81)	107.5 (78)	90.6 (77)	98.6 (78)
Treated	59.1 (117)	180.6 (116)	165.7 (112)	127.6 (114)	171.8 (110)

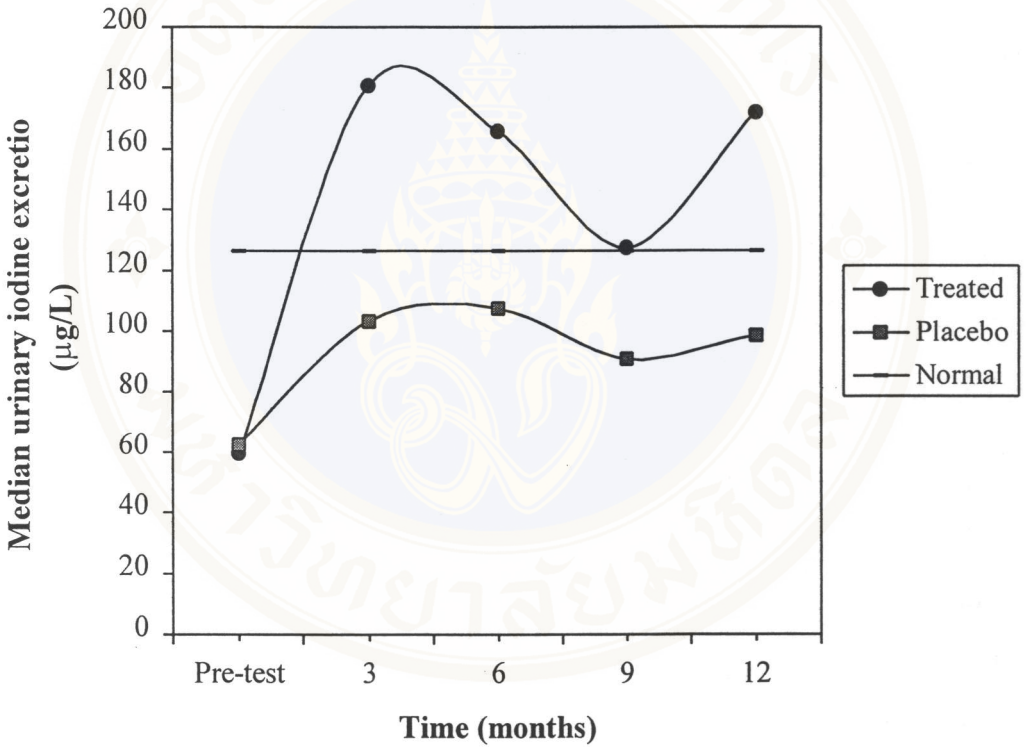


Fig. 10. Median urinary iodine excretion in treated, placebo and normal schoolchildren aged 6-14 years of various times

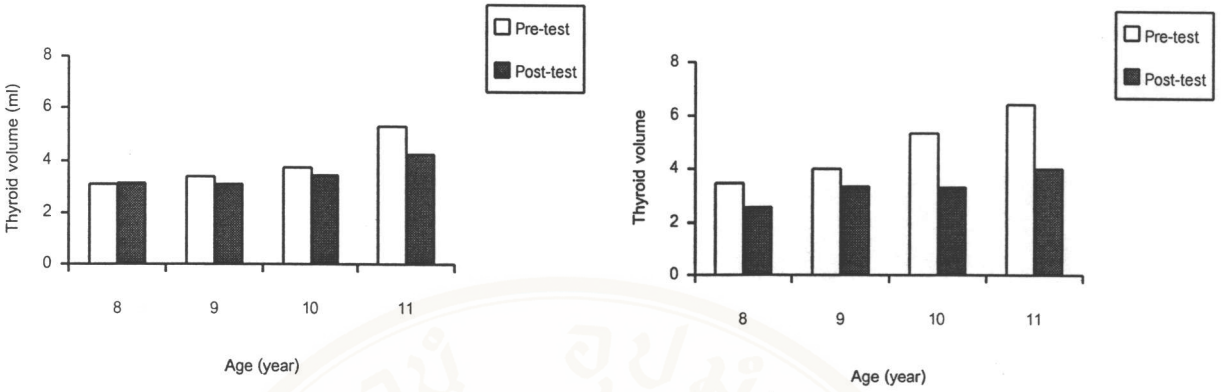
Thyroid volume by ultrasonography

The mean thyroid volume of schoolchildren aged 8-11 years of pre-test were compared to post-test in both treated and placebo group (Table 12.). In treated, there was significant difference between pre- and post-test of aged 8 yrs. ($P<0.01$), 9 yrs. ($P<0.01$), 10 yrs. ($P<0.001$)and 11 yrs. ($P<0.05$).

Pre-test, the mean thyroid volume in all ages of treated were higher than placebo (Table 12.). One year after receiving iodating water of 150 μgI/day, the thyroid volume in all ages were significant smaller than those of pre-test ($P< 0.05$ to 0.0001). The mean thyroid volume of placebo aged 8 and 11 years in post-test were nonsignificant smaller than those of pre-test ($P>0.05$). Significant smaller than those of pre-test in placebo was found in aged 9 ($P<0.02$) and 10 years ($P<0.05$).

Table 12. Thyroid volume of schoolchildren aged 8-11 years in Khamcha-I schoolchildren before and after receiving water without iodine in placebo and iodating water 150μgI/day in treated for one year.

Subjects	Age	Mean ± SD (n)		P-value
		Pre-test	Post-test	
Placebo				
	8	3.08 ± 0.65 (11)	3.12 ± 0.89 (11)	0.791
	9	3.37 ± 1.00 (50)	3.10 ± 1.13 (50)	0.018
	10	3.74 ± 1.01 (39)	3.42 ± 0.88 (39)	0.048
	11	5.29 ± 1.28 (11)	4.25 ± 1.18 (11)	0.095
Treated				
	8	3.48 ± 1.10 (17)	2.56 ± 0.75 (17)	0.002
	9	3.98 ± 1.85 (26)	3.32 ± 1.55 (26)	0.007
	10	5.32 ± 2.25 (36)	3.30 ± 1.20 (35)	0.000
	11	6.39 ± 2.86 (6)	4.02 ± 1.49 (6)	0.046



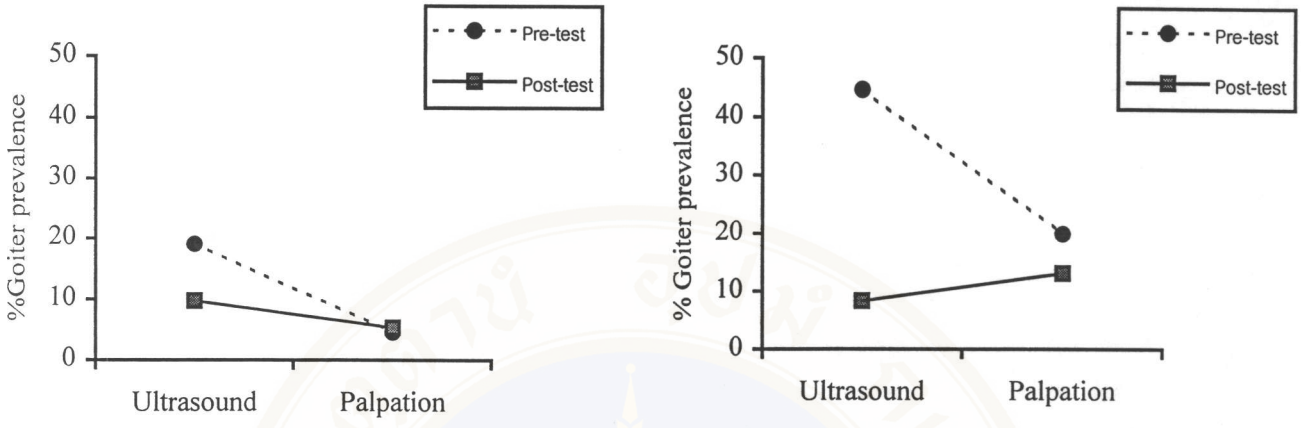
(a) Placebo (b) Treated

Fig. 11. Thyroid volume in pre-test and one year post-test of placebo (a) and treated group (b)

Table 13. Prevalence of goiter (%) by ultrasonography and palpation in schoolchildren aged 8-11 years at Khamcha-I district before and one year after oral iodated water 150 μgI/day.

	Goiter prevalence (n)	
	Placebo	Treated
Pre-test		
Ultrasonograph (P97)	19.08 (131)	44.71 (85)
Goiter palpation	4.58 (131)	20.00 (85)
Post-test		
Ultrasonograph (P97)	9.73 (113)	8.33 (84)
Goiter palpation	5.31 (113)	13.09 (84)

Table 13. and Fig. 12. shows the results obtained for goiter prevalence in treated and placebo using upper limit of normal thyroid volume (P97) compared to palpation. In pre-test the prevalence of goiter by ultrasound was 25 of 131 schoolchildren (19.08%) in placebo and 38 of 85 schoolchildren (44.71%) in treated. Thyroid gland enlargement by palpation was found in 6 of 131 schoolchildren (4.58%) from placebo and in 17 of 85 schoolchildren (20.00%) from treated.



(a) Placebo (b) Treated

Fig. 12. % Prevalence of goiter in pre-test and one year post- test by ultrasound and palpation in placebo (a) and treated group (b)

In post-test, the prevalence of goiter was decreased to 9.73% (11 of 113 schoolchildren) in placebo and 8.33% (7 of 84 schoolchildren) in treated by ultrasound. Enlargement of thyroid by palpation was found in 6 of 113 schoolchildren (5.31%) from placebo and in 11 of 84 schoolchildren (13.09%) from treated.

In iodine deficient area there is a preferential production of less iodinated thyroid hormone, with change in the ratio between serum T_3 and T_4. The mean ± SD of serum T_3, T_4 and TSH in treated of pre-test were 131.27 ± 36.28 ng/ml, 7.50 ± 1.61 μg/dl and 2.59 ±1.36 mIU/L respectively, and in post-test were 159.14 ± 49.75 ng/ml, 8.46 ± 1.97 μg/dl and 2.77 ± 1.39 mIU/L respectively (Table 14). The post-test results of T_3 and T_4 of treated were significant higher than those of pre-test ($P<0.001$). However these thyroid and thyroid related hormones level of pre-and post-test in both

treated and placebo were with in the normal. No significant difference of serum findings between pre-and post-test was observed in placebo (P>0.05).

From sera finding in treated group, it was found that 4 out of 147 cases were subclinical hypothyroidism in pre-test. In pos-test, there was all of 4 cases of improve subclinical hypothyroidism. 4 cases of new subclinical hypothyroidism from 130 normal in pre-test were obtained. In placebo, 2 out of 68 cases were subclinical hypothyroidism in pre-test. In post-test, there was no case of subclinical hypothyroidism was also observed.

Table 14. Sera findings in placebo and treated group.

	Mean ± SD (n)		
	Pretest	Post-test	P-value
Placebo			
T_3 (ng /dl)	132.51 ± 36.67 (67)	143.69 ± 32.38 (52)	0.098
T_4 (μg /dl)	7.93 ± 1.51 (68)	8.38 ± 1.73 (52)	0.054
TSH (mIU/L)	2.64 ± 1.31(68)	2.55 ± 1.16 (51)	0.602
Treated			
T_3 (ng /dl)	131.27 ± 36.28 (147)	159.14 ± 49.75 (136)	0.000
T_4 (μg /dl)	7.50 ± 1.61 (147)	8.46 ± 1.97 (138)	0.000
TSH (mIU/L)	2.59 ± 1.36 (139)	2.77 ± 1.39 (139)	0.057

Sera of anti-microsomal and anti-thyroglobulin abtibodies in pre-test of both treated and placebo were all negative. In post-test, anti-Tg antibodies titre of 1:10 and 1:40 were found 2 cases and 1 case of treated group respectively. Anti-microsomal antibodies titre of 1:1,600 was found only 1 case from treated. 1 case of positive anti-microsomal antibodies (1:1,600) in placebo was found.

From serum thyroglobulin findings in table 5 it was found that there was nonsignificant difference between placebo and treated in pre-test. Serum

thyroglobulin levels in 24 of 77 cases were higher than the normal value was 0-12 ng/dl (placebo 7 and treated 17 cases). These subjects were 6 boys and 18 girls.

Goiter grades by ultrasound and serum Tg concentration were shown in Table 15 It was found that serum Tg level of goiter grade 0 (5.80 ± 3.50 ng/dl) was lower than grade 1 (8.63 ± 7.57 ng/dl). It is indicated that measurement of serum Tg in serum can grossly mirror the size of the thyroid in regions where IDD is found (14).

Table 15. Thyroglobulin level in various goiter grades by ultrasonography.

Goiter grade	n	Serum Thyroglobulin (ng/dl) Mean ± SD
O	5	5.80 ± 3.50
1	38	8.63 ± 7.57

The correlation between thyroid volume and thyroglobulin in Mukdaharn was also calculated ($r = 0.348$, $P<0.05$, $n = 52$). There was no correlation between TSH and uriny iodine, and between Tg and uriny iodine.

CHAPTER V

DISCUSSION AND CONCLUSION

From the studies the several important findings were found in Khamcha-I district, Mukdaharn province. Firstly, Quantitative determination of thyroid gland volume was more accurate for the diagnosis of goiter grade than palpation. Pre-test: the prevalence of goiter in placebo and treated were 4.58% and 20.0% by palpation, 19.08% and 44.71% by ultrasound respectively. Post-test: the incidence by ultrasound was decreased to 9.73% and 8.33% in placebo and treated respectively. When compared with palpation, the incidence in treated was also decreased to 13.09%, but slightly increased to 5.31% in placebo. The discrepancy between ultrasound and palpation were 14.5% (placebo) and 24.7% (treated) in pre-test and 4.42% (placebo) and 4.76% (treated) in post-test. These results confirming that palpation are relatively inaccurate for assessing the prevalence of goiter in both severe and mild IDD. It is indicated that ultrasonography should be the method of choice in IDD area. Palpation can be applied to assess the prevalence of goiter in iodine sufficient area which goiter rate is lower than 5%.

Normal thyroid volume in Bangkok schoolchildren aged 8, 9, 10 and 11 years (338 boys and 304 girls) was also performed. Mean of normal thyroid volume in Bangkok schoolchildren was slightly higher than median value in all ages. The upper normal limit (P97) was a continuous increase with age. Non-significant difference between right and left of thyroid gland, boys and girls were observed. There was a

weakly positive correlation between thyroid volume and age (r = 0.311, n = 507), body weight (r = 0.389, n = 492), height (r = 0.440, n = 492) and body surface area (r = 0.432, n = 492). The best correlation was between thyroid volume and height.

Due the time consuming, the thyroid volume in normal schoolchildren aged 8, 9, 10 and 11 years were calculated by using transverse scan. The thyroid volume was obtained by multiplying width and thickness with the optimised correlation factor (cf) = 1.099, 1.224, 1.455 and 1.376 respectively. There was non-significant difference in thyroid volume of schoolchildren between our formula in all ages ($P > 0.05$). It is suggested that ultrasonography by our formula should be studied more in schoolchildren aged 4-6 and 12-15 years. This formula could be the method of choice in the future. In addition, it is noninvasive, rapid, precise and without discomfort to the patient.

Serum findings

In treated: the post-test results of both T_3 and T_4 were significant higher than those of pre-test ($P < 0.001$). Serum TSH was not significant higher than pre-test ($P > 0.05$). In placebo: There was non-significance of serum findings between pre-and post-test ($P > 0.05$). However serumT_3, T_4 and TSH concentration of pre-and post-test in both groups were within the normal limit. It is suggested that serum TSH will be useful indicator for congenital hypothyroidism.

Serum anti-microsomal and anti-Tg antibodies in pre-test of both groups were all negative. In post-test: 3 cases of low titre of anti-Tg antibodies were found in treated. Positive cases of anti-microsomal antibodies were found in both groups.

Mean ± SD of serum Tg of goiter grade 0 (5.80 ± 3.50 ng/dl) was also lower than grade 1 (8.63 ± 7.57 ng/dl). It is indicated that measurement of serum Tg in

serum can grossly mirror the size of the thyroid in area where IDD is found. The correlation coefficient (r) between Tg and thyroid volume was 0.348 ($P<0.05$, $n = 52$). There was no correlation between TSH and urinary iodine and between Tg and urinary iodine.

Urinary iodine excretion in primary schoolchildren was more sensitive indicator for determination of IDD than serum TSH concentration in both treated and placebo. The iodine prophylaxis program for IDD using iodated water should be continued. Monitoring and evaluation of IDD by palpation and urinary iodine excretion in these primary schoolchildren should be done once a year.

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