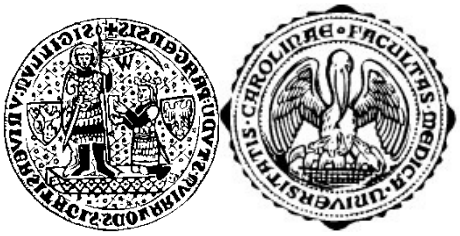

Charles University in Prague
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INVESTIGATION OF THYROID DISORDER IN PREGNANCY AND REFERENCE INTERVALS IN EVALUATION OF MATERNAL THYROID FUNCTION

(PhD Thesis Summary)

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1. Contens

2. Summary

The importance of maternal thyroxine for the development of the fetus brain early in pregnancy has received increasing acceptance. It has more recently become evident that maternal hypothyroxinemia results in the birth of children with decreased mental and psychomotor development.

In our group of 7,530 women in 9-11 week of pregnancy were determined TSH, anti TPOAb and FT4. For evaluation of results was necessary to set reference intervals for pregnant women.

The TSH reference interval was determined to be 0.06 - 3.67 mU/l and for FT4 9.8 - 23.43 pmol/l was used. The limit for anti-TPO positivity was determined to be 143 kU/l.

A raised concentration of TSH was found in 5.14% of women; and a suppression of TSH was found in 2.90% of women and 11.5% of pregnant women were found positive.

Serum concentrations of FT4 were lower in TPOAb positive as compared to TPOAb negative women and differences of FT4 in euthyroid women with suppressed, normal and elevated TSH were found.

In the study group there was no significant ($p<0.05$) difference in TSH or anti-TPO concentration in the groups, separated by age.

Physical examination, family and personal history and laboratory data were analysed in 318 from 1205 women with any positivity (TSH higher than 3.67 or lower than 0.06 mIU/l, positive anti TPOAb), and thyroid ultrasound (TUS) was realised. In this positive group had been diagnosed 60.4 % women with subclinical hypothyroidism, 4.7 % with manifest hypothyroidism and 21.7 % had suppressed TSH - most probably owing to a high concentration of hCG. The next finding was 2.8 % of thyreotoxicosis and also two cases of adenomyosis and one papillary carcinoma of thyroid gland. In all, 67.9 % of positive women were treated by any medication. 82.6 % of women treated for hypofunction were anti-TPO positive.

In Czech Republic, case finding screening is able to disclose only about 20% of asymptomatic mild or deep hypothyroidism or women with positive anti TPO in pregnancy. Foreign study report 70% of these high-risk pregnant women, consequently is evident need of general screening of pregnancy thyroid failure in Czech Republic. Moreover the investigation of TSH, FT4 and anti TPO together is necessary.

3. Abbreviation

anti -TPO	thyroid peroxidise antibodies
anti TG	thyroglobulin antibodies
$FT4_4$	free thyroxine
free β hCG	free beta subunit of hCG
hCG	human choriogonadotropin
IQ	intelligence quotient
M	mean
Mdn	median
N	number
NACB	the National Academy of Clinical Biochemistry
PAPP–A	pregnancy–associated plasma protein A
SD	standard deviation
T3	trijodtyronin
T4	thyroxin
TG	thyreoglobulin
TSH	thyroid stimulating hormone
TUS	thyroid ultrasound
VZP	General Health Insurance Company

4. Introduction

Many changes in the functioning of the thyroid gland occur during pregnancy and some diseases of the thyroid gland can affect both the pregnant woman and the fetus. Hypothyroidism is the most serious disorder of those occurring during pregnancy, and it might go unnoticed as some 'nonspecific' problem. Pregnant women with subclinical hypothyroidism seem to escape early clinical detection. The implications are staggering when one considers that there is a significant increase in intrauterine deaths, spontaneous abortions, premature births, and pre-eclampsia; also the development of the fetus, such as major malformations and loss of IQ. While the hyperfunction during pregnancy usually manifests itself by clinical symptoms or a relapse of a previously cured disease (mostly Graves - Basedow), lowered functioning is much more dangerous because of its non-specific symptoms. It has been clearly proven that even slight (subclinical) hypothyroidism affects not only the course of pregnancy, but (especially later-on) the neuropsychological development of the child. Symptoms of hypothyroidism (fatigue, lowered performance, sleepiness, psychological lability) can also accompany the physiological pregnancy; some women with subclinical hypothyroidism are absolutely asymptomatic and there is no reliance on the clinical image, while diagnostic of functional failure.

Results of surface population screenings are slightly varied, depending upon on level of medical care and approach to prevention, geographical conditions, supplementing with iodine, and other circumstances (including used diagnostic criteria). Evaluating thyroidal function during pregnancy is difficult, considering the other differing influences of pregnancy.
Determining TSH in the serum is a basic searching procedure in the diagnosis of function of the thyroid gland in the general population. Its regulation is based on feedback, however during pregnancy there are also other mechanisms taking place - mainly there is suppression of TSH, presumably due to a slight increases in the free thyroxin concentration driven by high hCG values.

By using the classical reference range for serum TSH one might misdiagnose as healthy those women who already have a slight TSH elevation and, conversely, one might suspect hyperthyroidism in normal women who have a lowered serum TSH value.

Determining the reference range suitable for the 1st trimester of pregnancy is possible by using the suggestion of the National Academy of Clinical Biochemistry (NACB) on selected samples from the population, or as 95th percentile from the cohort of women.

Determining FT4 is by watching the amount of biologically active hormone which is available to the organism of a pregnant woman (as well as the fetus), and is not affected by the concentration of binding proteins. Its concentration during pregnancy is partly effected by inflow of iodine and the duration of the pregnancy. Some consider it even more informative than TSH during pregnancy. During the 1st trimester, the fetus is completely dependent upon thyroxin produced by the mother. Even a small unnoticed malfunction of the thyroid gland, which doesn't have to endanger the course of the pregnancy, can affect the psychomotor development of the child. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny.

Anti-TPO antibodies are markers of autoimmune process in the thyroid gland, their determination is diagnostically and prognostically important. Presence of anti-TPO during pregnancy also alerts to the danger of development of postpartum tyreoiditidis; about 50% of anti-TPO positive women have some thyroid dysfunction after delivery, so it is necessary to follow-up these women.

The recommended dietary allowance for nonpregnant, nonlactating women aged ≥14 year is 150 µg/d. The iodine requirement during pregnancy is sharply elevated because of an increase in maternal thyroxine production to maintain maternal euthyroidism and to transfer thyroid hormone to the fetus, iodine needs to be transferred to the fetus for fetal thyroid hormone production in later gestation and a probable increase in renal iodine clearance. The recommended dietary allowance for pregnancy is 220 µg/d. In Czech Republic has been iodized salt in regular use since the 1950s, a good level of iodine supplementation can be expected also on Zamrazil (2004) study.

Malfunction of the thyroid gland during pregnancy is long-term, and still not a sufficiently solved problem. On many pages of scientific literature and specialist literature there are still new arguments to systematically screen pregnant women for thyroid underfunction and asymptomatic chronic thyroiditis in order to give such women the appropriate treatment.

5. Aims

The aim of this study was to introduce an estimation of thyroid dysfunction during pregnancy, selection of suitable analytic methods and determination of reference intervals for these markers in pregnancy.

1. The selection of efficient combination of methods for detection of thyroid dysfunction and the suitable term for investigation during pregnancy. Assess the interferences of other parameters, especially effect of high level of hCG on TSH.
2. Determination of the reference intervals for selected markers in the first trimester of pregnancy in plentiful group of pregnant women.
3. Defined reference intervals use to assign the percentage of thyroid dysfunction in the population of pregnant women who are undergoing their first trimester prenatal screening of the Down syndrome. These women devolve on endocrinologist and especially pregnant women with anti-TPO positivity follow up also postpartum.
4. Verify statement about increased incidence of thyroid dysfunction in older pregnant women.

6. Material and Methods

6.1 Studied groups

The study group (nonselected-NS) consisted of 7,350 pregnant women (in their 9^{th} – 11^{th} week of pregnancy, 99% Caucasian) who were undergoing their first trimester prenatal screening in the Institute of Clinical Biochemistry and Laboratory Diagnostics of Charles University, Prague, Czech Republic. Blood samples were collected non-contactly and centrifuged for 10 min after temperature reduction and formation of the clot. The serum was removed and after free β hCG and PAPP-A (for first trimester screening) determination were serum assayed for TSH and anti-TPO. FT4 was determined only in the case of TSH or anti-TPO out of the reference interval. The exact iodine status of this study group is not known.

All participating pregnant women gave informed written consent wit this subsequent investigation.

1) For evaluation of the reference interval, a selected group (**S1**) was created in accordance with the recommendations of the National Academy of Clinical Biochemistry (NACB). From the group of pregnant women, all those with a history of thyroid disease were excluded; anti-TPO > 60 kU/l (the manufacturer's cut-off) and free β hCG higher than triple of the median (Mdn=56.6 μg/l), in view of the suppression of TSH by a high level of hCG, were also excluded. Data for the other recommendation of the NACB - a family history of thyroid disease for the selection of women – were not accessible.
2) For the determination of pregnancy cut-off for anti-TPO, a selected group (**S2**) was created. From all of the women, women with a known history of thyroid disease were separated, as well as those with TSH lower than 0.06 and higher than 3.67 mU/l our new reference interval for TSH in the first trimester of pregnancy.
3) The entire nonselected group was divided into separate subgroups by age. The Tukey test was applied for determination of the statistical significance of differences with TSH and anti-TPO levels. The control group **(C)** for the evaluation of mean age was created by using pregnant women with TSH between 0.06 - 3.67 mU/l (our new reference interval) and with anti-TPO under the manufacturer's reference interval of 60 kU/l.

6.2 Immunoanalytical methods

6.2.1 Determination of TSH, anti-TPO, and FT4

were assayed by ADVIA® Centaur™ (Siemens), with chemiluminometric detection. TSH was determined by sandwich imunoanalysis with direct chemiluminometric technology; for anti-TPO and FT4 competitive imunoanalysis with direct chemiluminometric technology was used.

Reproducibility of this method is expressed by the interassay variability. For TSH, it is 5 - 7% for levels of 0.43 – 15.00 mU/l; for anti-TPO it is 10% for the level of 70 kU/l and 7% for the level of 170 kU/l. Interassay variability for FT4 is 7 - 9% for levels 10.1 - 33.0 pmol/l.

6.2.2 Determination of free β hCG

Free β hCG was assayed on a BRAHMS KRYPTOR. This system uses the TRACE (Time Resolved Amplified Cryptate Emission) technology, based upon a non-radiative transfer of energy. This transfer takes place between two fluorescent tracers: a donor (europium cryptate) and an acceptor named XL665 (a phycobilisome obtained from red algae). In the immunometric assay both are bound to an antibody.

Interassay variability for free β hCG is 3.0%

6.3 Statistical evaluation

The statistical analysis was performed using Statistica 7.1 CZ software (StatSoft, Prague 6, Czech Republic). All values are reported as mean±standard deviation or as shift percentages.

TSH and anti-TPO do not follow a normal distribution, they have to be normalized using log transformation. The reference interval was than determined using this log transformed data and geometric means are used to describe the means.

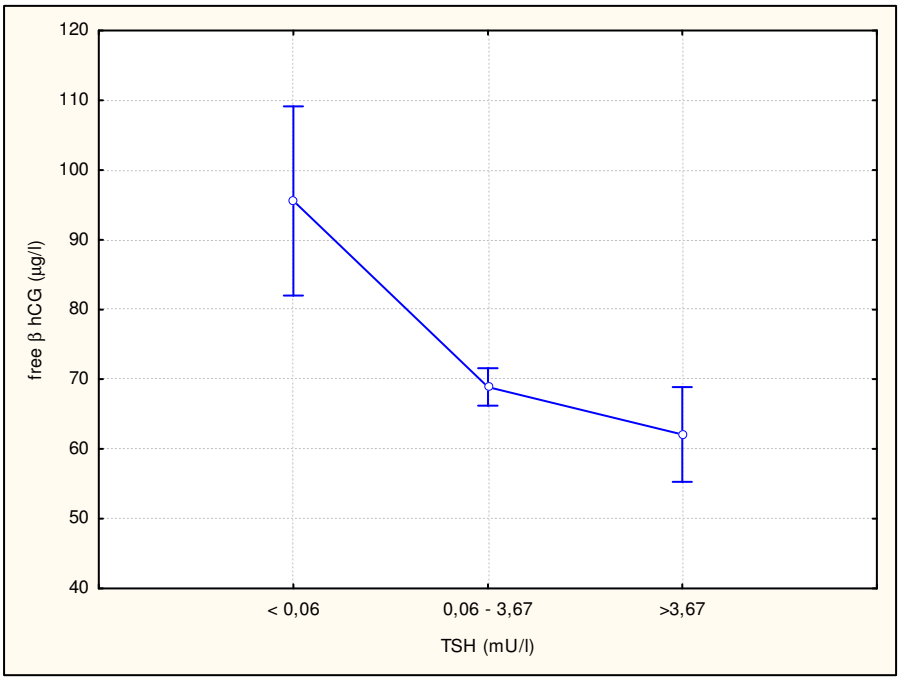
One way ANOVA and post-hoc p-levels for the Tukey honest significant difference (HSD) were considered to be statistically significant at the $p < 0.05$ level.

7. Results

7.1 Suppress of TSH level by high hCG

The mean free β hCG of women with suppressed TSH is double, in comparison with the group with a normal or higher TSH. One way ANOVA test at the $p< 0.05$ level was statistically significant for differences between the mean of free β hCG, for groups with different TSH levels. The results of the statistical analysis are shown in Figure 1. On the other hand, differences between groups with normal and higher TSH are not statistically significant.

Figure 1 Free β hCG in groups with different TSH level.



Human chorionic gonadotropin (hCG) free β-subunit measurement is used together with pregnancy associated protein A (PAPP-A) as a screening test for Down syndrome during first trimester of pregnancy. Difficulties arise mainly from low stability of free β-hCG subunit. Our study of stability free β hCG in real condition of collection and transport blood samples shows that results are reliable, when samples of free β hCG are separated immediately, (max. 4 h after drawing) stored at 2 – 8^{o}C and analyzed within 2 days.

7.2 Reference intervals and decision limits in first trimester of pregnancy

7.2.1 Determination of TSH reference interval

The nonselected group (NS), as well as the selected group S1, were statistical analyzed, and results for all years together are shown in Table 1. Group S1 was composed of pregnant women with no history of thyroid disease, anti-TPO level lower than 60 kU/l and free β hCG lower than triple of the median (Mdn=56.6 μg/l)

An acceptable reference interval for TSH in pregnant women was selected like 2.5th percentile for lower and 97.5th percentile of the selected group S1 (0.06 - 3.67 mU/l). A very similar upper limit was founded by using the 95th percentile of the nonselected group (NS) (3.71 mU/l).

Table 1 Reference ranges of TSH in pregnancy for nonselected (NS) and selected (S1) groups of pregnant women

TSH mU/l	**N**	**Median**	**Min.**	**Max.**	**2.5th percentile**	**5th percentile**	**95th percentile**	**97.5th percentile**
NS	5520	1.280	0	411.874	0.048	0.147	3.713	4.796
S1	4337	1.213	0	11.534	**0.062**	0.154	3.144	**3.670**

7.2.2 Determination of FT4 reference interval

FT4 was determined only for women (n=1176) with TSH lower than 0.1, and higher than 3.00 mU/l (or anti-TPO higher than 60 kU/l). For FT4 we used our reference interval of 9.8 - 23.43 pmol/l the manufacturer's one (9.8 - 23.1 pmol/l) for all populations. Statistical evaluation of FT4 results is shown in Table 2.

Table 2 Statistical evaluation of FT4

N	mean	Median	Min.	Max.	2,5th percentile	97,5th percentile
1176	14,77	14,13	4,67	44,46	9,8	23,43

Serum concentrations of FT4 were lower in TPOAb-positive group of pregnant women (median 13.79 pmol/l), in TPOAb-negative group were median 15.18 pmol/l These differences was significant on P<0.001. The median of FT4 level in euthyroid women with suppressed TSH was 17.89 pmol/l, with normal TSH was 13.98 pmol/l and in group of women with elevated TSH was median 12.91 pmol/l. The differences was significant at P<0.05.

7.2.3 Determination of anti TPO cut-off

The selected group S2 (pregnant women with no history of thyroid disease and with TSH level between 0.06 and 3.67 mU/l), as well as nonselected group, were statistical analyzed and the results for all years together are shown in Table 3.

Table 3 Cut-off of anti-TPO antibody for nonselected (NS) and selected (S2) groups of pregnant women.

Anti TPO (kU/l)	**N**	**Median**	**Min.**	**Max.**	**5th percentile**	**95th percentile**	**90th percentile**
NS	5520	38	0	15000	8	908	196
S2	5281	37	0	15000	8	577	**143**

The decision limit for the recommendation to visit an endocrinologist was determined to be 143 kU/l anti-TPO.

7.3 Detection of thyroid diseases in pregnancy in Czech population

Evaluation of positive results for the TSH reference interval created at the 2.5th, 5th, 95th and 97.5th percentile of the selected group S1 (S1 – pregnant women with no history of thyroid disease, anti-TPO level lower than 60 kU/l and free β hCG lower than triple of the median (Mdn=56.6 μg/l) are presented in Table 4.

In our group of pregnant women, 2.90% had a low level of TSH; 5.14% had TSH over the reference interval.

Table 4: Distribution of TSH in groups of women in the 1st trimester of pregnancy by different reference interval (percentile of selected group S1).

	TSH mU/l	**N**	**%**
< 2.5th percentile	**< 0,062**	214	**2,9**
< 5th percentile	< 0,154	378	5,14
> 95th percentile	> 3,144	592	8,05
> 97.5th percentile	**> 3,670**	378	**5,14**

The selected group (S2), as well as nonselected group, were statistical analyzed and the results with anti-TPO positivity for each year as well as all together are shown in Table 5.

Using the manufacturer's cut-off level we found 20.6% of the women to be with positive anti-TPO antibodies; using our decision value (143 kU/l) it was 11.5%

Table 5: Anti-TPO positivity for manufacturer's cut-off and for our pregnacy cut-off

anti TPO (kU/l)	N > 60 kU/l	%	N > 143 kU/l	%
All	1506	20,6	851	**11,5**
2005	289	32,11	99	11
2006	356	27,77	157	12,25
2007	377	16,85	237	10,59
2008	456	17,96	338	11,76
2009	28	19,36	20	10,26

There were 35 (0.48%) women with FT4 under and 28 (0.37%) women with FT4 over the reference range. The mean level in the group in reference interval of FT4 was 14,55 ±2,44 pmol/l.

7.3.1 Positivity in study group of pregnant women

Physical examination, family and personal history and laboratory data were analysed in 318 from 1205 positively screened women (TSH>3.67 mIU/l and/or positive TPOAb), and thyroid ultrasound was realised.

Women with any positivity of thyroid dysfunction were informed and half of them visited the endocrinologist within 1-4 weeks, the rest between 8-20 weeks. Structure of diagnosis in group of 318 women which were follow up in hospital endocrinology is in the Table 6.

Table 6: Structure of diagnosis in group of positive women

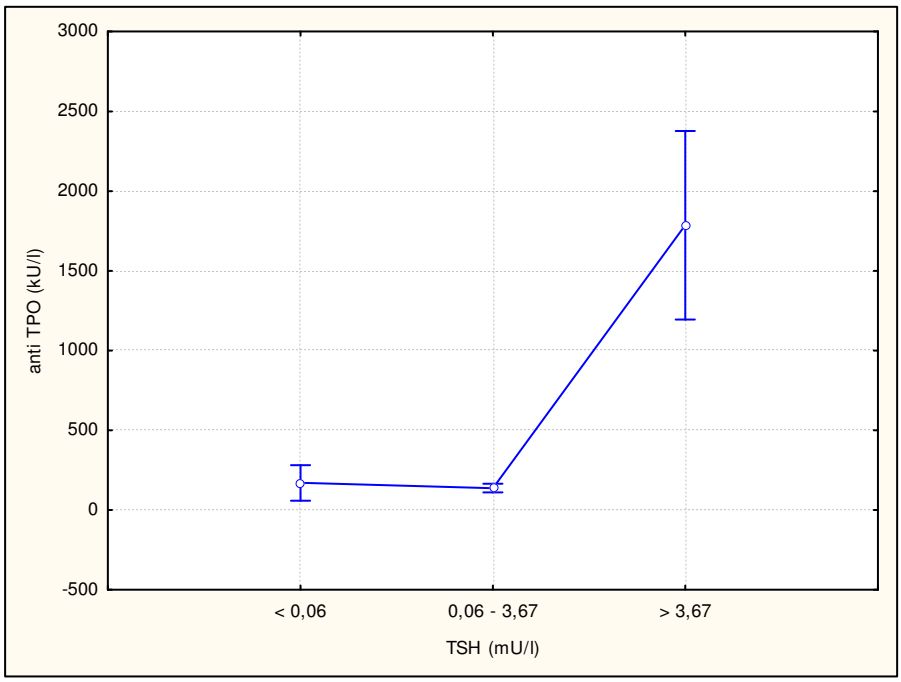
subclinical hypothyroidism	60,4%
overt hypothyroidism	4,7%
hyperthyroidism	2,9%
thyroid cancer	2,8%
without malfunction	7,5%
low TSH without malfunction	21,7%

There was no difference in TPOAb levels between women with positive and negative TUS. All women with elevated TSH were asymptomatic. Family and/or personal history of thyroid diseases and/or autoimmune diseases were present in 21.9 % of them.

7.3.2 Anti-TPOAb positivity and TSH level

Higher TSH is also very often associated with positivity of anti-TPO antibodies. In the group with TSH > 3.67 mU/l there were 44.1% of women with antibody positivity (anti-TPO > 143 kU/l); and in groups with TSH <0.06 mU/l, or in the pregnancy reference range only 10.1% or 9.1% of the women are with antibody positivity. One way ANOVA test at the $p < 0.05$ level was statistically significant for differences between the levels of anti-TPO for groups with different TSH level. Level of anti-TPO in the group with higher TSH is significantly higher at $p < 0.050$; differences between groups with low (or normal) TSH are not statistically significant and there are showed on Figure 2.

Figure 2: Anti TPO in groups with different TSH level



7.4 The influence of age over anti-TPO antibodies positivity and high TSH

The average age in the noneselected group (NS) was 31.3 (+/- 4.6); in the control group (C) of pregnant women it was 31.2 (+/- 4.3) years. One way ANOVA analysis confirmed no statistically significant differences in the average age, at the $p < 0.05$ level, between groups with different TSH and anti-TPO levels.

The average age in the groups with different TSH and anti-TPO levels are shown in Table 7.

Table 7: Mean age of pregnant women by different TSH and anti-TPO concentrations

TSH (mU/l)	**N**	**mean age**	**SD**		**anti-TPO (kU/l)**	**N**	**mean age**	**SD**
<0.06	158	31.4	0.451		< 60	4470	31.1	0.081
0.06 - 3.67	5096	31.1	0.075		60 - 143	462	31.1	0.230
>3.67	266	30.9	0.289		143 - 1000	344	31.6	0.300
					> 1000	244	31.3	0.328

There was no significant trend ($p<0.05$) for TSH or anti-TPO levels to rise with increasing age in the women.

8. Discussion

8.1 Suppress of TSH level by high hCG

Suppressed serum TSH concentration during gestation follow hyperthyroidism as well as hyperemesis gravidarum or high hCG levels. Lower serum TSH in pregnancy is influenced by the thyrotropic activity of elevated circulating human chorionic gonadotropin concentrations, mainly in the first trimester. In study group of pregnant women with suppressed TSH the average level of hCG was almost double (M=95.6 mg/ml) in comparison with group with TSH in reference range (M=68.9 mg/ml) or with TSH >3.67mU/l (M=62.1 mg/ml). Differences between the normal and raised TSH groups in hCG levels were not significant at $p<0.050$. In 1990, Glinoer and his associates examined distributions of maternal TSH measurements throughout pregnancy.They documented a downward shift in TSH levels during the first trimester – attributable to a weak TSH-like effect of hCG – followed by a gradual rise as pregnancy proceeded. The pattern of TSH measurements described in this study for the first trimesters is consistent with his work. The other authors also confirm that sub-normal serum TSH levels in the first trimester are coincident with rising hCG levels.

8.2 Reference intervals and decision limits in first trimester of pregnancy

Determination of the specific reference intervals for TSH, FT4, and anti-TPO during pregnancy is one of the basic requirements when implementing a general examination of the thyroid gland in early pregnancy. Reference intervals for different methods and manufacturer's may vary, they have been established using pools of nonpregnant normal sera and with different antibodies. Such reference ranges are not valid during pregnancy. A reference interval is the range of values of a test result for a defined population. The range is usually specified as the central 95% of the values, from the lower 2.5% to the upper 97.5% of the population. In older references the reference interval is often designated as the reference range.

For determination of reference intervals various methods are used. Haddow (2004) used for TSH the 98^{th} percentile in the entire group or anti-TPO negative woman. This cut-off resulted to 4.3% of first trimester women being identified as high risk. To achieve intended target of 2% (based on clinical correlation), they would need to set the cut-off higher. Vaidya (2006) used reference range based on the squared 95% confidence intervals in anti-TPO negative woman for TSH and FT4. TSH and anti-TPO have to be normalized using log transformation, as they do not follow a normal distribution. The National Academy of Clinical

Biochemistry (NACB) guidelines are commonly used and TSH reference intervals are established from the 95% confidence limits of the log-transformed values of anti TPO Ab-negative, ambulatory, euthyroid subjects who have no personal (or family) history of thyroid dysfunction and no visible goiter.

The manufacturer's reference range for in laboratory used TSH method (ADVIA:Centaur Siemens) is 0.37 - 5.00 mU/l. For the 1st trimester, the specific reference range was used the 2.5th and 97.5th percentile of the selected group S1, without anti-TPO positive and woman with hCG > triple of the median. The reference ranges for TSH were established upon 0.06 - 3.67 mU/l. When was used, for evaluation, the 95th percentile in the group of nonselected samples, the upper limit was 3.71 mU/l. Vaidya (2006) calculated for the TSH reference range 0.09 - 3.03 mU/l, in comparison to manufacturer's interval 0.27 - 4.20 mU/l. The other authors apply the manufacturer's or their own reference range for TSH, which vary from 2.0 to 5.0 mU/l. Serum TSH values are dependent on the method used and each laboratory should establish a trimester-specific reference range for pregnant women.

FT4 levels fit a Gaussian distribution, so reference ranges were derived using nonparametric analyses such as the 95th percentile. The calculated reference interval 9.8 - 23.43 pmol/l was very similar to the manufacturer's interval of 9.8 - 23.0 pmol/l. In this study was measured FT4 only in case of positivity anti-TPO, or if TSH was out of the reference range. It is recommended to keep the level of FT4 in pregnancy over half of the reference range.

Differences in anti-TPO antibody manufacturer's reference range are incomparable: from 0.5 to 100 kU/l. NACB stresses that the reference intervals for thyroid peroxidase antibodies (anti TPO) should be based on young men who lack certain risk factors and have serum TSH between 0.5 and 2.0 mIU/L. In this study was calculated the reference interval at the 90th percentile from the selected group S2, with a TSH level in new established pregnancy specific range of 0.06 – 3.67 mU/l. The anti-TPO positivity cut-off was established to 143 kU/l.

8.3 Detection of thyroid diseases in pregnancy in Czech population

In the group of 7, 350 women, 213 (2.90%) had their TSH under the reference interval (<0.06 mU/l). The prevalence of hypertyreosis in pregnant women is 1.7%, and 0.4% of these women had an elevated serum FT4 level. This is similar to that reported for non-pregnant individuals.

Many authors have determined the prevalence of hypothyreosis (overt and subclinical) in pregnancy and it is estimated to be 0.3 - 0.5% for overt hypothyroidism and 2 - 3% for subclinical hypothyroidism. In this study there were 4.5% of pregnant women with TSH over reference interval (>3.67 mU/l); which is in concordance with previous hypotheses, as well as with Haddow (2004) who used their own reference interval, identifing 4.3% of pregnant women with high TSH levels.

The positivity of anti-TPO in nonpregnant individuals is about 11%; in the pregnant population it is very similar. Negro (2006) mentioned 11.7% anti-TPO positive pregnant women; Dossiou (2008) selected groups by age and the positivity was 10.4 and 12.6 for ages 25 and 35 years, respectively. When was used the reference interval recommended by the producer of reagents (> 60 kU/l), there was 22.1% positivity. If was used the 90^{th} percentile (143 kU/l) as the cut-off for our group of pregnant women, there was 11.5% positivity in group with no age differentiation. These results correlate very well with other studies. Pregnant women with anti-TPO between 60 and 143 kU/l were given notice to visit an endocrinologist after delivery; women who had anti TPO > 143 kU/l were invited to do so immediately.

There exists a positive association between the presence of thyroid antibodies and pregnancy loss with postpartum thyroiditis.

Presently available information that supports the hypothesis that an inappropriate first trimester surge in maternal FT4, whatever the circulating TSH, would interfere with the development of the cerebral cortex, even if maternal euthyroidism is maintained by normal circulating T3. There is at present increasing consensus (Morreale, 2004) that maternal hypothyroidism, both clinical and subclinical, requires early detection and prompt treatment, because of its important negative effects for the woman, the pregnancy and the child.

The most practical approach is to screen all pregnant women for hypothyroidism as early in pregnancy as possible (or before conception). In the case of the mother, screening would reset in early diagnosis and treatment of subclinical hypothyroidism. Unfortunately, pregnant women with subclinical hypothyroidism seem to escape early clinical detection. In Mitchel (2004) study, 58% of the hypothyroid women were unaware of their disorder, and it took a median of 5 years from the time of the pregnancy for the clinical diagnosis to be made.

Vaidya (2007) study shows that targeted thyroid function testing of only high-risk pregnant women would miss nearly one-third of women with overt/subclinical hypothyroidism during early pregnancy. In Czech Republic, case finding screening is able to

disclose less than 20% of asymptomatic mild or deep hypothyroidism or women with positive TPOAb in pregnancy.

The relationship between anti-TPO and TSH is not definite, despite it being known that women with high level of TSH more frequently have positive anti-TPO antibodies. In study group, divided by serum TSH concentration, were 44.1% anti-TPO positive (in part), with TSH >3.67 mU/l and 10.1% or 9.15% in the group with TSH < 0.06 mU/l or TSH in the reference interval. Glinoer (1990) also documented somewhat higher TSH levels among the sub-population of women with elevated antibody levels and these findings are confirmed in the present study.

8.4 The influence of age over anti-TPO antibodies and high TSH

The greater the pregnant women's age very often is a reference risk factor of thyroid malfunction. Stricker (2007) published that the anti-TPO level was related to maternal age. Negro (2006) presented a higher prevalence of anti-TPO positivity in older women, and the NHANES III study shows slightly increasing levels of TSH and anti-TPO with age. In contrast to these, in this study was did not find a significant ($p < 0.05$) difference between the average age of the pregnant women with positive anti-TPO and those with low TSH (or in the reference interval). There is also no significant ($p<0.05$) difference in TSH or anti-TPO concentration in groups, separated by age. It is possible that this is because 76% of those pregnant women were within the ages of 25 - 35.

An answer to the question of screening cost-effectiveness of thyroid function in pregnancy was already presented by Dosiou (2008). In our study is not defined cost-efficiency, but it is unquestioned fact that early diagnosis of thyroid disorder is cost-effective and beneficial for both mother and child.

Maternal hypothyroxinemia appears to be a much more frequent cause of deficits in the progeny than congenital hypothyroidism, for which we have successful neonatal thyroid screening programs. This study maybe will help define the impact of universal screening (TSH, FT4, anti-TPO) on the health care system. The other analysis would be more clearly identify the causal relationships between mild thyroid hormone deficiency and thyroid autoimmunity, on the one hand, and fetal neurological development on the other. In the meantime, physicians and obstetricians must use their own judgment about the optimal management for their individual patients.

9. Conclusion

1. In the group of 7,350 women (in their 9^{th} – 11^{th} week of pregnancy) who were undergoing their first trimester prenatal screening were serum assayed (with informed consent) for TSH, anti-TPO, resp. FT4. Determining TSH in the serum is a basic search procedure in the diagnosis of the thyroid gland function. Determination of FT4 is by watching the amount of biologically active hormone which is available to the organism of a pregnant woman (as well as the fetus), and is not affected by the concentration of binding proteins. Anti-TPO antibodies are markers of autoimmune process in the thyroid gland, the presence of anti-TPO during pregnancy also alerts one to the danger of development of postpartum tyreoiditidis.

FT4 concentration during pregnancy is partly effected by both the inflow of iodine and the duration of the pregnancy. TSH regulation is based on feedback; however, during pregnancy there are also other mechanisms taking place - mainly suppression of TSH, presumably due to the thyroid-stimulating activity of hCG early in pregnancy when hCG levels are highest. We studied the stability of free β hCG in maternal blood upon storage and during sample transport. Serum for Down syndrome screening should be separated till 2 hours after collection, stored at 2 – 8^{o}C and analyzed within 2 days.

The post-hoc analysis of our results by application of the Tukey HSD test showed statistical significant differences between means of free β hCG in the group with low TSH (< 0.06 mU/l), and in groups with normal or higher TSH. Lower TSH level in the first trimester is significant only if other markers (FT4 or anti-TPO) are out reference interval too.

2. For the right evaluation of our results we determined the reference intervals for TSH, anti TPO, and FT4 in the first trimester of pregnancy. TSH and anti-TPO do not follow a normal distribution, they have to be normalized using log transformation. An acceptable reference interval for TSH in pregnant women **(0.06 - 3.67 mU/l)** we selected like 2.5^{th} percentile for lower and 97.5^{th} percentile of the group S1 (pregnant women with no history of thyroid disease, anti-TPO level lower than 60 kU/l and free β hCG lower than triple of the median Mdn=56.6 μg/l). In this study was calculated the decision limit for anti-TPO at the 90^{th} percentile from the selected group S2 (pregnant women with no history of thyroid disease and with TSH level between 0.06

and 3.67 mU/l),with a TSH level in new established pregnancy specific range of 0.06 – 3.67 mU/l. The anti-TPO positivity cut-off we established to **143 kU/l.** FT4 levels fit a Gaussian distribution, so reference ranges we derived using nonparametric analyses such as the 95th percentile. The reference interval was **9.8 - 23.43 pmol/l**.

3. In all our study group 7,350 women were 1205 with any positivity. There were 2.90 % women with low and 5.14 % with high TSH; 0.38 % with high and 0.48 % with low FT4. Positivity of anti-TPO antibodies in all of them was 11.5 %. About this abnormality we informed their attending physician, with approach to cooperative endocrinologist.

   318 of this 1205 women with TSH lower then 0.06 mU/l or higher then 3.67 mU/l or wit anti-TPO positivity were attending the 3rd Medical Department - Clinical Department of Endocrinology and Metabolism of General Teaching Hospital Prague.

   Subclinical hypothyroidism was diagnosed in 60.4%, overt hypothyroidism in 4.7%, hyperthyroidism in 2.9% and thyroid cancer in 2.8% of 318 women. There was no difference in TPOAb levels between women with positive and negative TUS. All women with elevated TSH were asymptomatic. Family or personal history of thyroid or autoimmune diseases were present in 58.3% all of them. Women with subclinical hypothyroidism had family or personal history of thyroid or autoimmune diseases only in 21.9% of them.

4. World guidelines for management of thyroid dysfunction during pregnancy and postpartum recommend not universal but only case finding screening. Our study strongly support the need of establishing mass screening programs for pregnant women early in gestation in the Czech Republic. Screening would result in early diagnosis and treatment of thyroid disease, and it is possible to combine with first trimester screening for Down's Syndrome.
5. In this study we did not find a significant difference between the average age of the pregnant women with positive anti-TPO and those with low TSH (or in the reference interval). There is also no significant difference in TSH or anti-TPO concentration in groups, separated by age. It is possible that this is because 76% of our pregnant women were within the ages of 25 - 35.

In 2009 is proving the system general testing of the insufficient function of the thyroid gland during early pregnancy by TSH, FT4 and anti-TPO levels determination in some parts of Czech Republic. This project is supported by the VZP (General Health Insurance Company)**,** the biggest health insurance company in the Czech Republic.

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