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Evaluation of the thyroid function of healthy pregnant women by five different hormone assays

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A normal function of the thyroid gland during pregnancy is essential. Any change can affect both the pregnant woman and the fetus. Thyroid hormones play a crucial role in the brain development of the fetus, thus proper maternal free thyroid hormone levels are important especially during the first trimester. We compared the free thyroid hormone levels FT3 and FT4 in forty pregnant women with no thyroidal disease by five different assays available on the market. The blood samples were collected between the 8th and 22nd weeks of pregnancy. The correlation coefficient "r" between different assays was 0.908–0.975 for TSH, 0.676–0.892 for FT4 and 0.480–0.789 for FT3. These data show that the inter-assay results varied widely in the studied population. One reasonable explanation may be that during pregnancy the serum levels of the thyroid hormone binding proteins are altered and "free" hormone measurements by immunoassays are influenced by these alterations. Thus, the results may show higher or lower thyroid hormone values depending upon the assay used. Therefore, it is strongly suggested that every laboratory should establish its own pregnant reference ranges for the tests used for the evaluation of thyroid function, based on values of the population served.

1. Introduction

Pregnancy is a physiological state of the female body during which many changes in the function of different organs occur. This is true for the production and serum levels of the thyroid hormones as well, and the evaluation of test results is often a challenge. The major question is when these changes should be considered pathological. It is well known that some of the diseases of the thyroid gland can affect not only the pregnant woman's health but also the development of the fetus. Thus, it is crucial to differentiate between the physiological alterations of the thyroid function in pregnant women, and those changes that need medical attention or treatment.

Hypothyroidism is the most common disorder of the thyroid gland that can develop during pregnancy. It might easily remain unnoticed or its clinical signs may be misinterpreted as normal signs of pregnancy. However, there is a significant increase in intrauterine deaths, spontaneous abortions, premature births and pre-eclampsia (Casey et al. 2006; Lazarus et al. 2002, 2005; Poppe et al. 2007; Springer et al. 2009). It can negatively affect the development of the fetus, resulting in major malformations and decrease in IQ (Haddow et al. 1999; Smallridge et al. 2001; Klein et al. 2001). In contrary, the hyperfunction of the maternal thyroid during pregnancy usually manifests with clear clinical symptoms, an example being the relapse of a previously cured Graves' disease (Abalovich et al. 2007). One can conclude that hypothyroidism is more dangerous because of its insidious onset and non-specific symptoms. It has been clearly proven that even mild, subclinical hypothyroidism affects both the course of pregnancy (Casey et al. 2006; Poppe et al. 2007) and the neuropsychological development of the child (Escobar et al. 2004; Mitchell et al. 2004; Pop et al. 2003). The well-known symptoms of hypothyroidism like fatigue, lowered performance, sleepiness, and psychological sensitivity can also accompany normal pregnancy; some women with mild hypothyroidism are absolutely asymptomatic (Abalovich et al. 2007).

While screening the population of a specific region, the results may slightly vary upon different factors such as the geographical conditions, iodine supplementation, level of medical care (Abalovich et al. 2007; Zimmermann and Delange 2004). Considering a smaller specific subpopulation, the evaluation of the thyroid function in pregnancy becomes even more difficult, since many influences may affect the outcome (Dayan et al. 2002).

The well-accepted assessment of the thyroid function can be achieved by measuring the human thyrotropin (TSH) and the free fractions of the thyroid hormones triiodothyronine (FT3) and thyroxine (FT4). The total T3 and T4 levels are not used any more for the evaluation of the thyroid function in general, and have been shown to produce misleading results in pregnancy. For screening purposes the measurement of the TSH is enough in most of the cases since a normal TSH level excludes any abnormality of the thyroid axis except the extremely rare case of pituitary TSH dysfunction. The regulation of the TSH is based on feedback mechanism; however, during pregnancy other mechanisms are involved as well. During the first trimester, a

suppression of the TSH occurs which is attributed to the action of human choriogonadothrop hormone (hCG) which has a high structural homology with the TSH. The peak hCG elevation and the nadir in serum TSH occur together at 10–12 weeks of pregnancy (Baloch et al. 2003). As a consequence, measuring only the TSH and evaluating the result using the reference interval established for the general population may be misleading in women with (inappropriately) high-normal TSH level. On the other hand, we might (mistakenly) suspect thyroid hyperfunction in the subpopulation having lowered TSH values (Glinoer et al. 1995).

However, the hormone changes during pregnancy have other effects as well. It is well-known that more than 99% of the T4 and T3 are bound to TBG and the other binding proteins, albumin and transthyretin. The elevated production of the estrogen is increasing the mean serum thyroxine-binding globulin (TBG) (Baloch et al. 2003). With abnormal TBG level total T3 and T4 (TT3 and TT4, respectively) levels are affected (Demers et al. 2003). Specifically during pregnancy, the TBG increases by 2 to 3 times compared to the pre-pregnancy level by the 20th week of gestation (Glinoer et al. 1990). This elevation in TBG results in an elevation of the TT3 and TT4 by an average of 1.5 times compared to the pre-pregnant levels early by the 16th gestational week (Pedersen et al. 1993; Nohr et al. 2000). The TT3 and TT4 measurements were overshadowed during the last decades by determination of the FT3 and FT4 fragments mainly because TT3 and TT4 present great variability and are highly dependent on the levels of the binding proteins. This is particularly true for the hormonal changes that occur during gestation.

The decreased TSH during the first trimester is associated by an increase in the FT4 level (Glinoer et al. 1990). This elevated FT4 level in a minority of cases can reach supranormal values and rarely may lead to the so called "gestational transient thyrotoxicosis" (GTT) characterized by symptoms of thyrotoxicosis and hyperemesis gravidarum (Jordan et al. 1999; Goodwin et al. 1992). During the second trimester FT4 and FT3 levels decrease and this decline can be even further amplified by the insufficient iodine supplementation of the mother in some cases (Glinoer et al. 1990). At times the FT4 level my fall below the reference limit for the normal population. The frequency of such low FT4 concentration is method-dependent (Roti et al. 1991). Those pregnant women who where already on levothyroxine (L-T4) substitution therapy before pregnancy usually require an increased amount of hormone in order to achieve normal serum TSH levels. Considering these hormonal changes, TSH and FT4 should be assessed together during monitoring levothyroxine substitution therapy in pregnant women. The L-T4 dose should be adjusted to obtain normal TSH and FT4 concentrations.

FT4 represents the amount of biologically active fraction of the hormone that is available to the body. During the first trimester of pregnancy, the assessed portion of thyroid hormone is the amount achievable not just for the mother but for the fetus as well. It is well known that the fetus is absolutely dependent on the mother's thyroxin production during the first third of gestation (Lazarus 2002). As mentioned above, even a little change in the function of the maternal thyroid that does not necessarily affect the course of pregnancy may affect the psychomotor development of the fetus (Escobar et al. 2004).

Any dysfunction of the mother's thyroid can have long-term consequences for the woman and especially the fetus and later the newborn. This emphasizes the importance of screening of the thyroid function very early during the first trimester of pregnancy. However, it is still not clear what reference range and which method should be used for the evaluation of the thyroid function in pregnancy. Also, there is doubt whether TSH alone or TSH and FT4 together should be assessed in every case.

Table 1: Correlation of the TSH levels measured by different assays (n = 40)

	AxSYM	Elecsys	LIAISON	LIAMAT	Vitros
AxSYM	1	0,936	0,927	0,928	0,942
Elecsys	–	1	0,966	0,916	0,975
LIAISON	–	–	1	0,918	0,941
LIAMAT	–	–	–	1	0,908
Vitros	–	–	–	–	1

Table 2: Correlation of the FT4 levels measured by different assays (n = 40)

	AxSYM	Elecsys	LIAISON	LIAMAT	Vitros
AxSYM	1	0,892	0,853	0,676	0,799
Elecsys	–	1	0,892	0,811	0,868
LIAISON	–	–	1	0,806	0,825
LIAMAT	–	–	–	1	0,755
Vitros	–	–	–	–	1

Table 3: Correlation of the FT3 levels measured by different assays (n = 4)

	AxSYM	Elecsys	LIAISON	LIAMAT	Vitros
AxSYM	1	0,541	0,517	0,575	0,480
Elecsys	–	1	0,789	0,741	0,596
LIAISON	–	–	1	0,670	0,660
LIAMAT	–	–	–	1	0,569
Vitros	–	–	–	–	1

The aim of the current study was the comparison of thyroid function of healthy pregnant women by five different automated analytical methods available on the market. Furthermore, we investigated whether the known elevation of TBG during pregnancy affects the results.

Also, we addressed the question if serum TSH measurement alone is suitable for the evaluation of the thyroid function during gestation. Finally, we examined whether the obtained results matched the clinical state of the investigated patients.

2. Investigations and results

The study group consisted of 40 pregnant healthy women with no known thyroid disease, in their 8th–20th week of pregnancy. The investigation was approved by the Institutional Ethics Committee. Before the procedures an informed consent was obtained from all pregnant women.

The measurement of TSH, FT3, and FT4 were performed by five different commercially available methods: LIA-mat S300, DiaSorin; LIAISON, DiaSorin; AXSYM, Abbott Laboratories; Elecsys 2010, Roche; Vitros ECi, Johnson&Johnson.

The statistical analysis was performed with the Anova program and the correlation coefficient "r" was calculated. Tables 1–3 show the TSH, F4 and FT3 results using the five laboratory methods. Table 4 presents the average TSH, FT4 and FT3 levels obtained by the different methods and the reference ranges according to the manufacturers' data.

The correlation between the results obtained by the different procedures has been weaker than expected. There was considerable variation, especially in case of FT3 measurements.

Table 4: Mean values measured by different assays and reference ranges

	LIAMAT	LIAISON	AxSYM	Elecsys	Vitros
TSH mU/L	1.074 (0.3–3.0)	1.122 (0.28–2.64)	1.163 (0.5–4.67)	1.722 (0.27–4.2)	1.250 (0.46–4.68)
FT4 pmol/L	9.920 (7.7–23.2)	12.064 (11.6–21.8)	12.309 (9.1–23.8)	16.010 (13–23)	14.868 (10–28.2)
FT3 pmol/L	4.364 (2.6–5.4)	3.667 (2.9–5.22)	3.708 (2.22–5.3)	5.865 (2.8–7.1)	7.120 (4.26–8.1)

For details of the tests see Section 4, Experimental. (n = 40)

3. Discussion

Reference ranges for TSH, FT4 and FT3 are usually defined for the general population, and for most of the general laboratory investigations, these ranges also apply to pregnant women. This is not true for thyroid function testing. The major causes are the regulatory changes. However, based on our data, methodological reasons are also important. Thyroid function reference ranges provided by the manufacturers are established using serum pools of non-pregnant women, and in most cases specific reference ranges applicable for pregnancy are not provided. Moreover, the results of TSH and free thyroid hormones may differ according to the investigated pregnant population, and are highly dependent on iodine supplementation and general nutritional status (Lazarus 2002).

For the determination of the reference intervals different methods have been used (Haddow et al. 2004). For the measurement of TSH Haddow used the 98th percentile for the pregnant women, while Vaidya suggested a range based on the squared 95% interval for TSH and FT4 (Vaidya et al. 2007). Some authors established trimester-specific reference ranges for the pregnant population (Soldin et al. 2004, 2007). Obviously, these data suggest a lower TSH interval for the first trimester. This is important for detection of pregnant women at risk of thyroid dysfunction. TSH values out of the trimester specific reference range warrant further endocrinological investigations, usually FT4 and FT3 measurements. The lower serum TSH concentrations observed in pregnancy are due to the elevated hCG level. Our data strongly support these notions (Table 4); the results for TSH are approaching the lower limit of the reference interval. The results slightly differ according to the laboratory methods; this is also true for the mean TSH values. The five different methods show a correlation between 0.91 and 0.98 for TSH (Table 1).

The results for the mean FT4 levels in the investigated healthy pregnant population shows even greater variability by the evaluated five laboratory methods, as demonstrated in Table 4; the correlation coefficient varied between 0.68 and 0.89 (Table 2). The differences between the results for the mean FT3 values were even more impressive (Table 4); the correlation coefficient being situated between 0.48 and 0.79 (Table 3). However, similarly to the results of others we also found that the fall in TSH during the first trimester of pregnancy was associated with a modest increase of the free hormone fractions (Glinoer et al. 1990).

In the present series of 40 healthy women in the 8th–22nd weeks of gestation, the five investigated laboratory methods showed a significant degree of variability of results as we have shown by evaluating the correlation coefficients between the tests. However, the average values were still situated within the reference ranges provided by the manufacturers.

In conclusion, we found notable differences in the results of the five commercially available hormone tests in the group of pregnant women. To avoid overlooking any sort of thyroid dysfunction in pregnant women, we recommend the measurement of both FT4 and TSH together in pregnancy. Also, it is strongly suggested that every laboratory should establish its own trimester specific reference ranges applicable for the specific tests used for the evaluation of thyroid function during gestation.

4. Experimental

The measurement of TSH, FT3, FT4 were performed by five different commercially available methods as follows:

4.1. TSH

1. LIA-mat S300, DiaSorin, second generation two-site immunochemiluminometric assay with two monoclonal antibodies, analytical sensitivity: < 0.02 mIU/L; 2. LIAISON, DiaSorin, third generation two-site immunochemiluminometric assay with two monoclonal antibodies, analytical sensitivity: 0.004 mIU/L; 3. AXSYM, Abbott, second generation, microparticle enzyme immunoassay with monoclonal and polyclonal antibodies, analytical sensitivity: 0.03 mIU/L; 4. Elecsys 2010, Roche, two-site immunochemiluminometric assay with two monoclonal antibodies, analytical sensitivity: 0.005 mIU/L; 5. Vitros ECi, Johnson&Johnson, third generation two-site immunochemiluminometric assay with two monoclonal antibodies, analytical sensitivity: 0.003 mIU/L.

4.2. FT4 and FT3

1. LIA-mat S300, DiaSorin, one-step simultaneous competitive chemiluminescence assay with SPALT monoclonal isoluminol-labelled antibody; 2. LIAISON, DiaSorin, one-step simultaneous competitive chemiluminescence assay with SPALT monoclonal izoluminol-labelled antibody; 3. AXSYM, Abbott, two-step sequential competitive microparticle enzyme immunoassay with alcalic-phosphatase labeled T3; 4. Elecsys 2010, Roche, one-step sequential competitive electrochemiluminescence assay with SPALT polyclonal rutenium-labelled antibody; 5. Vitros ECi, Johnson&Johnson, one-step simultaneous competitive chemiluminescence assay with SPALT monoclonal peroxidase-labelled antibody.

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References

Abalovich M, Amino N, Barbour LA, Cobin RH, De Groot LJ, Glinoer D, Mandel SJ, Stagnaro-Green A (2007) Management of thyroid dysfunction during pregnancy and postpartum: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metabol 92: 1–47.

Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR (2003) Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13: 3–126.

Casey BM, Dashe JS, Wells CE, McIntire DD, Leveno KJ, Cunningham FG (2006) Subclinical hyperthyroidism and pregnancy outcomes. Obstet Gynecol 107: 337–341.

Dayan CM, Saravanan P, Bayly G (2002) Whose normal thyroid function is better - yours or mine? Lancet 360: 353–354.

Demers LM, Spencer CA (2003) Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Clin Endocrinol 58: 138–140.

Glinoer D, De Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A (1990) Regulation of maternal thyroid function during pregnancy. J Clin Endocrinol Metab 71: 276–287.

Glinoer D, De Nayer P, Delange F, Lemone M, Toppet V, Spehl M, Grün JP, Kinthaert J, Lejeune B (1995) A randomized trial for the treatment of mild iodine deficiency during pregnancy: maternal and neonatal effects. J Clin Endocrinol Metabol 80: 258–269.

Goodwin TM, Montoro M, Mestman JH, Pekary AE, Hershman JM (1992) The role of chorionic gonadotropin in transient hyperthyroidism of hyperemesis gravidarum. J Clin Endocrinol Metab 75: 1333–1337.

Haddow JE, Knight GJ, Palomaki GE, McClain MR, Pulkkinen AJ (2004) The reference range and within-person variability of thyroid stimulating

hormone during the first and second trimesters of pregnancy. J Med Screen 11: 170–174.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchel ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ (1999) Maternal thyroid deficiency during pregnancy and subsequent europsychological development of the child. N Engl J Med 341: 549–555.

Jordan V, Grebe SK, Cooke RR, Ford HC, Larsen PD, Stone PR (1999) Acidic isoforms of chorionic gonadotrophin in European and Samoan women are associated with hyperemesis gravidarum and may |be thyrotrophic. Clin Endocrinol 50: 619–627.

Klein RZ, Sargent JD, Larsen PR (2001) Relation of severity of maternal hypothyroidism to cognitive development of offspring. J Med Screen 8: 18–20.

Lazarus JH (2002) Epidemiology and prevention of thyroid disease in pregnancy. Thyroid 12: 861–865.

Lazarus JH, Premawardhana LD (2005) Screening for thyroid disease in pregnancy. J Clin Pathol 58: 449–452.

Mitchell ML, Klein RZ (2004) The sequellae of untreated maternal hypothyroidism. Eur J Endocrinol 151: 45–48.

Morreale de Escobar G, Obregon MJ, Escobar del Rey F (2004) Role of thyroid hormone during early brain development. Eur J Endocrinol 151: 25–37.

Nohr SB, Jorgensen A, Pedersen KM, Laurberg P (2000) Postpartum thyroid dysfunction in pregnant thyroid peroxidase antibody-positive women living in an area with mild to moderate iodine deficiency: Is iodine supplementation safe? J Clin Endocrinol Metab 85: 3191–3198.

Pedersen KM, Laurberg P, Iversen E, Knudsen PR, Gregersen HE, Rasmussen OS (1993) Amelioration of some pregnancy associated variation in thyroid function by iodine supplementation. J Clin Endocrinol Metab 77: 1078–1083.

Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ (2003) Maternal hypothyroxinemia during pregnancy and subsequent child development: a 3 year follow – up study. Clin Endocrinol 59: 282–288.

Poppe K, Velkeniers B, Glinoer D (2007) Thyroid disease in female reproduction. Clin Endocrinol 66: 309–321.

Roti E, Gardini E, Minelli R, Bianconi L, Flisi M (1991) Thyroid function evaluation by different commercially available free thyroid hormone measurement kits in term pregnant women and their newborns. J Endocrinol Invest 14: 1–9.

Smallridge RC, Ladenson PW (2001) Hypothyroidism in Pregnancy: Consequences to Neonatal Health. J Clin Endocrinol Metabol 86: 2349–2353.

Soldin OP, Soldin D, Sastoque M (2007) Gestation specific thyroxine and thyroid stimulating hormone levels in the United States and worldwide. Ther Drug Monit 29: 553–559.

Soldin OP, Tractenberg RE, Hollowell JG, Jonklaas J, Janicic N, Soldin SJ (2004) Trimesterspecific changes in maternal thyroid hormone, thyrotropin, and thyroglobulin concentrations during gestation: trends and associations across trimesters in iodine sufficiency. Thyroid 14: 1084–1091.

Springer D, Zima T, Limanova Z (2009) Reference intervals in evaluation of maternal thyroid function during the first trimester of pregnancy. Eur J Endocrinol 160: 791–797.

Vaidya B, Anthony S, Bilous M, Shields B, Drury J, Hutchison S (2007) Detection of thyroid dysfunction in early pregnancy: Universal screening or targeted high risk case finding? J Clin Endocrinol Metabol 92: 203–207.

Zimmermann M, Delange F (2004) Iodine supplementation of pregnant women in Europe: a review and recommendations. Eur J Clin Nutr 58: 979–984.