Abstract

Background: Hashimoto’s thyroiditis is the principal aetiology of hypothyroidism with presence of anti-thyroperoxidase antibodies (anti-TPO). The association between anti-TPO and foeto-placental complications has been observed in previous studies. To go further in the understanding, the current study compares the level of anti-TPO in maternal blood and in the cord blood of her fetus at the moment of childbirth to demonstrate the passage of anti-TPO through the placental barrier.

Methods and Findings: This study was realised in a maternity ward located in the Northern district of Paris, France from 2006 to 2007. Women with normal pregnancy were included in a first study and only women with no abnormal thyroid dosage at baseline and tested positive with anti-TPO were prospectively enrolled. Maternal blood samples were collected in the third trimester and at the arrival to the ward when patients came to deliver. After delivery, cord blood sample was collected. Pearson’s correlation coefficient was computed. 5941 patients delivered in the ward during the study, 33 pregnant women were included. We found a correlation between the anti-TPO levels in maternal and in the cord blood of their fetus with a correlation coefficient of 0.98 and a p-value<0.001.

Conclusions: This is the first demonstration of the free passage through the placental barrier of anti-TPO from the mother to the fetus at the moment of childbirth. These findings can be extrapolated all along pregnancy and open the door to a direct action of the anti-TPO on fetus and to a possible action on the fetal thyroid.

Introduction

Hypothyroidism is one of the most common endocrine disorders [1]. Prevalence of hypothyroidism during pregnancy is from 1% to 2% [2–4]. Autoimmune disease is the principal etiology of hypothyroidism and particularly Hashimoto’s thyroiditis with anti-thyroperoxidase antibodies (anti-TPO) [5–7]. Hypothyroidism during pregnancy increases the risk to develop vasculo-placental complications [8,9] such as gravidic hypertension, pre-eclampsia, preterm, abruptio of placenta and post partum haemorrhage or post partum thyroiditis [10,11]. Fetal complications described are lower fetal birth weight and fetal distress [12]. However, the association between presence of anti-TPO, and such foeto-maternal complications have been observed [13,14] and some authors in literature believe that anti-TPO could play a role in vasculo-placental complications occurrence [15]. Otherwise, although maternal thyroid hormones do not pass easily placental barrier, they are essential for the formation of the nervous system from the beginning of gestation [16] as maternal iode transfer. The balance of thyroid hormones is measured by the level of free triiodothyronine (FT3), free thyroxine (FT4) and thyreo stimulating hormone (TSH) during pregnancy [17]. To go further in the understanding, the current study compares the level of anti-TPO in maternal blood to the level of anti-TPO in cord blood of her fetus at the moment of childbirth to demonstrate the passage of anti-TPO through the placenta barrier, and compares anti-TPO levels and TSH, FT4, FT3 values from the mother and her fetus at the same time.

Materials and Methods

This study is part of a first study [18] realised from 2006 to 2007 in the maternity ward of the pediatric hospital of Robert Debré Hospital located in the northern district of Paris, France. The study planned to prospectively recruit follow-up, maternal blood samples and cord blood samples of 110 pregnant patients receiving routine prenatal care. Inclusion criteria were normal pregnancy, having signed the study informed consent document, and being covered by the French public healthcare insurance system. The appropriate ethics committee (Comité de protection des personnes d’Ile de France) approved the study protocol may 12, 2005, under the number 051132. Exclusion criteria were the presence of chronic disease; iodine supplementation; current or past thyroid disease; fetal abnormalities; multiple pregnancy; pregnancy
induced using assisted reproductive technology, and abnormal thyroid hormone concentrations at baseline. Of 129 patients who were invited to participate in the study, 4 refused participation and 1 had abnormal serum thyroid hormone concentrations. Of the remaining 124 patients, 108 (87%) attended all the study visits. One patient had a miscarriage and another fetus died in utero at 22 weeks of gestational. Five additional patients asked to leave the study later during the pregnancy, usually at the request of the husband. All Pregnancy follow-up were realized in the ward and were seen monthly by an obstetrician. None of the study patients delivered prematurely. Cord blood samples were obtained at delivery from 72 fetuses from 72 pregnancies. Among these 72 pregnancies, were selected only women with no abnormal thyroid dosage at baseline tested positive with anti-TPO (Figure 1).

Maternal blood samples were collected in the third trimester and at the arrival to the ward when patients came to deliver. After delivery, cord blood sample was collected. All blood samples were tested after collection of all study data was complete. All sera were frozen at −80°C until use. Serum FT3, FT4, and TSH were assayed on an ACS-180 SE automate using chemiluminescence immunoassay. Variability was 3.3%, for FT3, 6.6%, for FT4, and 8.4% for TSH. The limits of detection were 0.3 pm/L, 1.3 pm/L, and 0.02 IU/L, respectively.

Serum levels of anti-TPO in maternal and cord blood were detected by direct chemiluminescence immunoassay on an ACS-180SE automate. The limit of detection was 15 UI/L.

Results are expressed as numbers and/or percentages for categorical variables and means ± standard deviation (SD), or median [min; max], for continuous variables, unless stated otherwise.

We compared hormonal levels between mother blood samples at the third trimester of gestation and fetal cord blood samples of their fetus. Each pregnancy was considered as an independent event. All quantitative variables were compared with non parametric Wilcoxon’s test. Pearson’s correlation coefficient was computed to assess the link between maternal and fetal thyroid parameters (FT3, FT4, TSH, and anti-TPO). All tests were two-tailed, p-values less than 0.05 were considered significant. Statistical analyses were performed using Statview Software V5 (SAS Institute Inc, Chicago, IL, USA; Copyright 1992–1998 Version 5.0).

Results

During the period of the study, a total of 5941 patients delivered in the institution. The 33 couples of blood samples from mothers with positive anti-TPO and cord blood samples of their fetus were matched for analyse in this study (Figure 1).

The median anti-TPO level in maternal blood was 47 UI/ml [28; 467] and, in cord blood 38 UI/ml [26; 417]. There was no significative difference between the levels of anti-TPO in the maternal blood and the fetus in cord blood. We investigated the correlation between the anti-TPO levels in maternal blood and in cord blood and found a strong correlation with a correlation coefficient of 0.98 and a p-value<0.001 (Figure 2).

The FT3, FT4, and TSH median levels were respectively 4.1 UI/ml [3.3; 4.8], 12.1 UI/ml [9.5; 15.5], and 1.39 UI/ml [0.59; 2.99] in maternal blood at the third trimester, 4.2 UI/ml [1.5; 4.8], 11.8 UI/ml [8.7; 15.2], and 2.47 UI/ml [0.22; 5.18] at delivery and 2.5 UI/ml [1.6; 4.5], 14.3 UI/ml [10.1; 16.7], and 7.4 UI/ml [0.5; 24.94] in cord blood (Figure 3).

We found a correlation between the FT3 and FT4 levels in maternal blood at delivery and cord blood with a correlation coefficient respectively of 0.4 and 0.4 with a p-values = 0.02 and 0.01. In contrary, for TSH, there was no correlation between levels in maternal blood and cord blood with a correlation coefficient of 0.32 and a p-value = 0.11. Finally, we searched for a correlation between the FT4 levels in maternal blood at third trimester and TSH levels in cord blood. We found no correlation with a coefficient of 0.32 and a p-value = 0.10 in the population of anti-TPO positive mothers (n = 26) but a strong correlation with a coefficient of −0.4 with a p-value = 0.006 in the initial population of the study (n = 36) (Figure 4).

Discussion

The main finding of this study is the first demonstration of the free passage through the placental barrier of anti-TPO from the mother to the fetus at the moment of childbirth with a strong correlation (p-value<0.001) and a “quasi linear” correlation coefficient of 0.98. These findings can not be reproduced during pregnancy for obvious ethical reasons but the characteristics of the placental barrier only slightly change after the first trimester of pregnancy and we can easily extrapolate these data all along pregnancy.

Hypothyroidism during pregnancy increases the risk to develop vasculo-placental complications such as gravidic hypertension (7.7%), pre-eclampsia (15.4%), preterm, abruptio placentae (3.8%), post partum haemorrhage (7.7%) and, post partum thyroiditis (19.2%) [10]. The effect on the fetus involving low birth weight (15.4%) and fetal distress had been recognised [12,22]. However, the association between the presence of anti-TPO, and such foeto-maternal complications has been observed [13] and many authors in literature believe that anti-TPO could play a role in the occurrence of these pathologies [15]. But so far, the pathogenicity of anti-TPO in pregnancy has not been clearly demonstrated. On the model of the demonstration of pathogenicity of antiphospholid antibodies [19,20], a study on pregnant mice showed after passive immunization with purified human IgG with anti-TPO activity [15] a lower fetal birth weight and placental weight. Immunohistochimic study showed deposits of IgG inside the wall of uterine vessels highlighting a possible vascular mechanism of action of anti-TPO. A second study conducted on

![Figure 1. Chart-Flow.](https://doi.org/10.1371/journal.pone.0084647.g001)
pregnant mice after passive immunization with recombinant mouse TPO, concluded to an increased fetal abortion rate related to circulating anti-TPO [21].

The demonstration of the passage of anti-TPO through the placental barrier is interesting on two points: first, it opens the door to a direct action of the anti-TPO on fetus and second to a possible action on the fetal thyroid. In fact, thyroid hormones are essential for fetal cerebral development [23,24]. The role of maternal thyroid status on the future neuropsychological development of the fetus is important at all stages of pregnancy [25]. The maternal hypothyroidism is potentially deleterious to the cerebral development of the fetus [26]. Thyroid autoimmunity is also noted to be associated with a significant increase in women with spontaneous miscarriages. Furthermore, although thyroid...
antibodies are decreased during pregnancy, thyroid function deteriorates into subclinical hypothyroidism in women with thyroid autoimmunity. Moreover, these women have TSH levels in the first trimester higher compared with cases of hypothyroidism without antibodies. This finding remains true throughout pregnancy until delivery with levels of FT4 30% lower compared with those of women who are antibody-negative [10]. Then, there could be an interest in screening for hypothyroidism around the pregnancy [27].

From 11 weeks of gestation, fetal thyroid can produce thyroid hormones [28] and TSH is detectable in fetal serum [29]. In second and third trimester, a materno-fetal gradient for thyroid hormone concentrations is found in literature with higher levels of FT4 and FT3 in maternal than in fetus serum, despite an increase in fetal FT4, and FT3. We found similar results for FT3 levels at delivery but not for FT4 levels (Figure 3). This could be partly explained by the small number of serum samples in our study and the fact that this gradient decreases while approaching the end of pregnancy when the fetal thyroid function evolves [30]. However, TSH levels still remain superior in cord blood than in maternal blood (Figure 3). This is due to the relative insensitivity of the pituitary gland of the fetus to the retro control of FT4 [31,32] but maybe also to the less sensitivity of fetal thyroid gland even if there is also placental transfer of FT3 and FT4 from the mother to the fetus, especially during late pregnancy [33].

Finally, in the population with anti-TPO positive mothers, there was no correlation between FT4 levels in maternal blood at third trimester and TSH levels in cord blood probably due to lack of power since in the entire population, we found a strong correlation. Theses findings suggest for the first time that the thyroid balance in mothers during pregnancy and particularly during third trimester could presume of thyroid activity in their fetus at delivery.

Conclusion

Many authors in literature believe that anti-TPO could play a role in the occurrence of foeto-maternal complications during pregnancy and therefore, there is an interest in screening for hypothyroidism around the pregnancy. We report the first demonstration of the free passage through the placental barrier of anti-TPO from the mother to the fetus at the moment of childbirth. These findings can probably extrapolate to the third trimester of pregnancy and open the door to a direct action of the anti-TPO on fetus and to a possible action on the fetal thyroid function.

Author Contributions

Conceived and designed the experiments: JS GA DL. Performed the experiments: JS GA JG DL. Analyzed the data: JS GA DL PFC. Contributed reagents/materials/analysis tools: JS GA JG DL. Wrote the paper: JS GA DL.

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Figure 4. Correlation between FT4 levels in maternal blood and TSH levels in cord blood at delivery. doi:10.1371/journal.pone.0084647.g004

Anti-TPO and Placental Barrier
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