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Thyroid hormone in Immunoglobulin Density (IgGp), and postpartum haemorrhage: two case reports

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Abstract
Background: The human foetus receives a passive immunization by selective passage across the placenta of maternal gamma immunoglobulin (IgG) by attaching to the constant fraction of neonatal receptor (FcRn) which is a specific IgG transporter. Two of the patients assayed hypothyroid had postpartum haemorrhage (PPH), incidentally also had very low mean thyroid hormone level which should normally increase in pregnancy.
Rationale/Aim: A probable relationship of immunoglobulin density (IgGp) of mothers, their thyroid hormone levels and postpartum haemorrhage was evaluated for possible clinical intervention.
Methodology: Twenty pregnant women were presented at our antenatal clinics and qualified for the inclusion criteria consented freely to participate in the investigation. Ex-vivo placental models of different thyroid states, as well as maternal blood from the antecubital vein and umblical cord blood (mixed blood) were taken within five minutes postpartum. The trafficking of IgG was investigated by immunohistochemical staining and pulse-chased at 37^0C at neutral pH. Maternal thyroid hormone levels were also evaluated.
Results: The IgG in two hypothyroid cases with post partum haemorrhage was significantly ($P<0.001$) deficient in the sera and ($P<0.01$) in the other hypothyroids that had no PPH when compared with euthyroid mothers.
Conclusion: The thyroid hormone level of the mother suggests to be an obvious natural determinant of the passive immunization of the neonate and possible occurrence of post partum haemorrhage.

Keywords: *Immunoglobulin density, Post-partum haemorrhage, thyroid hormones, hypothyroidism, immunization.*

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Résumé
Contexte : Le fœtus humain reçoit une vaccination passive par passage sélectif à travers le placenta de l'immunoglobuline gamma maternelle (IgG) en s'attachant à la fraction constante du récepteur néonatal (FcRn) qui est un transporteur IgG spécifique. Deux des patients hypothyroïdiens assayed ont eu l'hémorragie puerpérale (PPH), incidemment ont également eu le niveau moyen très bas d'hormone thyroïdienne qui devrait normalement augmenter dans la grossesse.
Justification / But : Une relation probable de densité d'immunoglobuline (IgGp) des mères, de leurs niveaux d'hormone thyroïdienne et de l'hémorragie puerpérale a été évaluée pour l'intervention clinique possible.
Méthodologie : Vingt femmes enceintes ont été présentées dans nos cliniques prénatales et qualifiées pour les critères d'inclusion qui ont consenti librement à participer à l'enquête. Des modèles placentéraux ex vivo de différents états thyroïdiens, ainsi que le sang maternel de la veine antecubitale et du sang de cordon ombilical (sang mélangé) ont été pris dans les cinq minutes du post-partum. Le trafic d'IgG a été étudié par coloration immunohistochimique et chassé à 370C au pH neutre. Des niveaux maternels d'hormone thyroïdienne ont été également évalués.
Résultats : L'IgG dans deux cas hypothyroïdiens avec l'hémorragie post-partum était significativement ($P<0.001$) déficient dans le sera et ($P<0.01$) dans les autres hypothyroïdes qui n'ont eu aucun PPH par rapport aux mères euthyroïdes.
Conclusion : Le niveau d'hormone thyroïdienne de la mère suggère d'être un déterminant normal évident de la vaccination passive du nouveau-né et de l'occurrence possible de l'hémorragie post-partum.

Mots-clés*: Densité d'immunoglobuline, hémorragie post-partum, hormones thyroïdiennes, hypothyroïdie, immunisation.*

Introduction

The constant fraction of neonatal receptor (FcRn) for gamma immunoglobulin (IgG) has been well characterized in the transfer of passive humoral immunity from a mother to her foetus. in addition, FcRn protects IgG from degradation, thereby explaining the long half-life of this class of antibody in the serum. In recent years, it has become clear that FcRn is expressed in various sites in adults, where its potential function is now beginning to emerge. In addition, recent studies have examined the interaction between FcRn and the Fc portion of IgG with the aim of either improving the serum half-life of therapeutic monoclonal antibodies or reducing the half-life of pathogenic antibodies.

The present work is a follow-up of a report on immunoglobulin density (IgGp) and Apgar score [1]. The human foetus receives a passive immunization by selective passage across the placenta, of maternal gamma immunoglobulin (IgG) [2], by attaching to the constant fraction of neonatal receptor (FcRn) which is a specific IgG transporter [3,4,5]. Receptors for the Fc region of IgG, FcRn, are an important bridge between antibodies and cellular effector systems [6]. A heterogenous but homologous group of FcRn integral membrane glycoproteins that are members of the immunoglobulin gene super family, is expressed on the surfaces of human leucocytes, monocytes, macrophages and platelets [7,8].

Experiments using binding of fluorescent-conjugated heat-aggregated IgG [1,9] to placental tissue sections revealed in the presence of FcvR on villous endothelial cells, mononuclear phagocytes (Hofbauer cells). Expression of this Fc receptor is not restricted only to the pre- or neonatal period when it plays a role in the delivery of maternal IgG to offspring. It can also be ubiquitously found in adult endothelial tissues where it is believed to be involed in IgG homeostasis [10]. The active transport seems to be controlled by the maternal level of IgG which thus stabilizes the foetal IgG level [2] and the neonatal Fc receptor (FcRn) is a specific IgG transporter [3-5,11].

Rationale

Some deliveries in the labour rooms were agonizing and protracted due to poor cervical dilation. Earlier work revealed that most hypothyroid subjects presented with prolonged bleeding, clotting and prothrombin times [12] and decreased muscle tone due to decrease muscle tone due to decrease serum calcium concentration [13]. There could be an insight into these agonizing deliveries that triggered the present investigation with respect to possible influence of thyroid hormone if any, on the association of IgGp with PPH and the hormone upon which possible interventions could be recommended.

Materials and methods

A total of twenty pregnant patients were selected, fifteen presenting at the Jos University Teaching Hospital (JUTH) and five at TADAM Medical Centre, Jos. Ten were primipara while ten were multipara. The Helsinki Declaration principles as revised was strictly followed, and the mothers who happened to be educated freely opted to participate in the exercise. Inclusion criteria were normal term pregnancy without complication, infection or disease or preeclampsia/eclampsia.

Methodology

Ex-vivo placenta of euthyroid and hypothyroid states were taken, as well as maternal blood from the antecubital vein and cord blood from the umbilical cord, within 1 - 5 minutes after delivery of the infant. Fluorescein dye was injected into the placental artery and the umbilical cord within 2 - 3 minutes of expulsion.

Trafficking: The trafficking of IgG was investigted, using a modified method of Daniel *et al* [6]. Immunohistochemical staining was done with anti FEA_1 (Sandoz, Basel, Switzerland) which is an early endosomal marker of human leucocyte endothelial cells (HULEC-5A) mononuclear cells that had been pulsed with Alexa 647-labelled $H_{435}A$. The cells were stained with this marker which tags to FcRn or IgG and pulses the IgG. All the immunostains were monoclonal antibodies (Mab) (British Drug House, Poole, England). Chasing the IgG by the fluorescence of the fluorescein dye, the fate of the migration of IgG following pinocytic uptake into microvasculature cells was for 20 - 40 minutes. Microvasculature-derived cells, (with the aid of a dissecting microscope) were used in preference to endothelial cells, isolated from large vessels, since FcRn is preferably expressed in mcro-vessels [6]. The microvasculature cells were "washed" and pulse-chased at 37°C in a medium depleted of serum IgG (BDH Chemicals Ltd. Poole, England) at pH 7.0. Pulsing at this pH precluded the possibility that IgG binds to cell surface FcRn prior to uptake; as FcRn-IgG interaction is not permissive at neutral pH [3].

Estimation of thyroid hormone: The mean thyroid hormone level in each mother's serum (euthyroid and hypothyroid groups) was estimated by Enzyme-Linked Immunosorbent Assay (ELISA) method; as

Table 1: Mean (X) IgG density (IgGñ) and thyroid hormone levels in neonates of hypothyroid and euthyroid mothers

	IgG Density in mothers		IgG Density in Neonates of		Maternal Serum T_4ng/dL	
	HM	EM	HM	EM	HM	EM
	0.25	11.0	2.5	10.0	0.6	3.8
	0.50	10.50	3.0	11.5	0.6*	4.0
	0.30	11.50	2.0	12.0	0.5**	3.5
	0.25	10.0	2.5	10.5	0.8	3.0
	0.25	10.0	2.5	11.0	0.5**	2.8
X ±	0.76 ±	10.70 ±	2.5 ±	11.0 ±	0.6 ±	3.45 ±
SEM	0.44	0.35	0.16	0.05	0.05	0.05

Key: HM = Hypothyroid Mothers, EM = Euthyroid Mothers
T_4 = Thyroid Hormone Level (n = 20)
***P<0.001*
**P<0.01*

specified by WHO International Laboratories (Kits) for Biological Standard London.

Principle: The ELISA is a commonly used analytical biochemical assay. The assay uses a solid-phase Enzyme Immunoassay (EIA) to detect the presence (qualitatively or quantitatively) of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured. In ELISA, various antigen-antibody combinations are used, always including an enzyme-labelled antigen or antibody and enzyme activity is measured using a substrate that changes colour when modified by the enzyme.

Statistics: The data collected were statistically analysed using ANOVA (SPSS Version 17) to assess the contribution of thyroid hormone in IgG population (IgGñ) and PPH.

Results

The results of mean and standard error of mean (SEM) of IgGñ of mothers and their neonates, as well as the T_4 levels of the mothers are shown in Table 1 and Plates 1, 2 and 3. Plate one shows a longitudinal section of umbilical cord and ex-vivo term placenta of euthyroid mother: IgG appears as greenish patches that are more on foetal side (f) than on maternal side (m) showing vectorial mobility. Plate 2 is a transverse section of ex-vivo term placenta of hypothyroid mother showing IgG as fluorescent green-white spots distributed more or less evenly on both maternal end (m) and foetal side (f) displaying scalar motility. Plate 3 is a transverse section of ex-vivo term placenta showing part of the vasculature (v) surrounding a central mucous area (m).

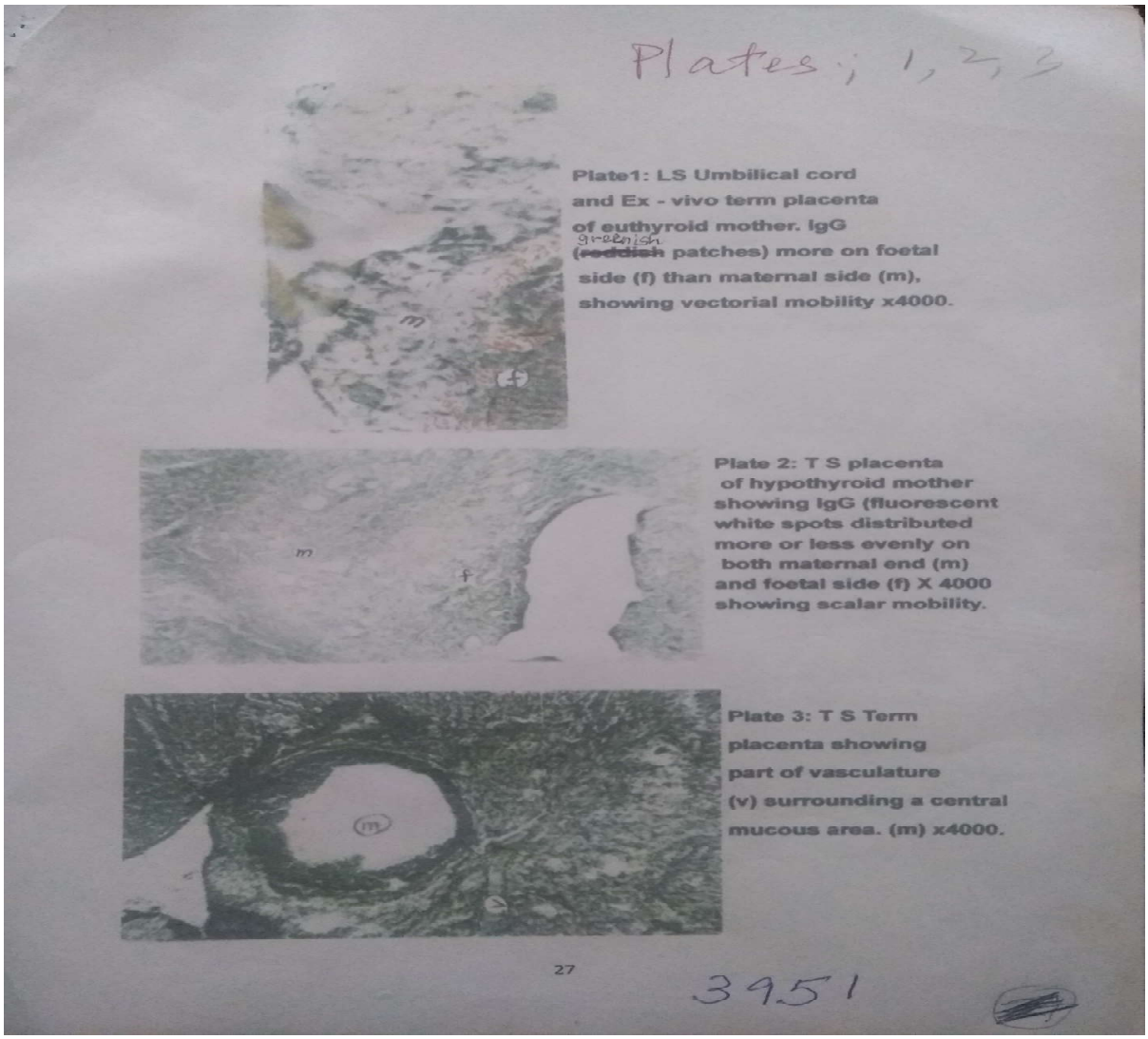
The IgG density (IgGñ) in post-partum haemorrhage (PPH) was significantly (P<0.001) depressed in sera of hypothyroid mothers. The mean physiological plasma thyroxine (T_4) level was found to be 3.45±0.05ng/dL of seum in 10 euthyroid cases studied. Two of the hypothyroid cases had post-partum haemorrhage and mean thyroid hormone (T_4) level of 0.5ng/dL, while the mean for the hypothyroid group was 0.6±0.05ng/dL. At the serum T_4 level of 0.5ng/dL there was post-partum haemorrhage in the two cases.

Discussion

In the current study, ex-vivo placenta, maternal and cord blood have been used to analyse the materno-foetal transfer of IgGp by determining the IgGñ in maternal and cord blood by tagging the antibody with fluorescein dye and comparing the population with thyroid hormone (T_4) levels in the sera of both euthyroid and hypothyroid participants. Our finding was that the IgGñ increased in umbilical cord blood in euthyroid women, but a sharp decline (P<0.01) was noted in hypothyroid mothers irrespective of mode of delivery, barring complications. It was however noted that the hypothyroid mothers had protracted dystocia lasting eight to ten hours as previously reported [1]. Two cases in the hypothyroid group had T_4 levels of 0.5ng/dL and post-partum haemorrhage (PPH); unlike other hypothyroids that had a mean T_4 level of 0.6ng/dL, but no PPH.

The striking phenomenon was that thyroid hormone level should normally increase in pregnancy but serum T_4 levels depreciated significantly and moreso in the two mothers who had PPH and thus the case reports of this investigation.

Euthyroid mothers had their babies per vaginal within two to four hours. On the other hand, hypothyroid mothers had very slow cervical dilatation, suggesting hypothyroidism as a factor affecting



duration of labour and the level of IgG in cord blood. It is generally assumed that the pressure of the uterine contraction during parturition leads to filtration of IgG into the foetal circulaton [14-16]. The strong uterine contraction in euthyroid mothers probably enhanced IgG filtration into the cord circulation, but the weak contractions of the hypothyroid myometrium would not filter much IgG across the materno-foetal circulation. Hypothyroidism was observed in the investigation to factor into PPH. Hypothyroidism has been reported earlier to cause feeble contractions [13,16,17] consequent on severe myotonia [13]. Thyroid hormone is known to switch on Ca^{2+} receptors and thus muscular atony coupled with pressure of the foetus on the cervix might have seeded the rupture of blood vessels and thus haemorrhage due to prolonged clotting, bleeding and prothrombin times [12].

Much as the resistance to the passage of the neonate through the birth canal filters IgG into the foetal circulation [14,15,16], the thyroid hormone level of the mother, from this work, appears to be an obvious natural determinant of the neonatal passive immunization (IgGñ) and the possible occurrence of PPH. The thyroid hormone status of the mother determines the vectorial transfer of materno-foetal IgG, the neonatal health and post-partum haemorrhage.

The findings in the present investigation suggests that T_4 levels below 0.6ng/dL, may, in addition to other negative pregnancy outcomes for both mother and neonate, lead to post-partum haemorrhage. This justifies our recommendation that thyroid hormone screening be incorporated into antenatal clinics to spare hypothyroid pregnant women the agony of protracted labour and post-partum haemorrhage, and as an intervention for the future of maternal cum neonatal health.

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