Imperial College
London

CLINICAL STUDY PROTOCOL

Full Title: A longitudinal study of alterations in metabolic markers and gut hormones in pregnant and non-pregnant patients with intrahepatic cholestasis of pregnancy, gestational diabetes mellitus and normal pregnant and non-pregnant controls

Short Title: The Metabolic Profile in intrahepatic cholestasis of pregnancy and gestational diabetes mellitus

Chief Investigator: Professor Catherine Williamson

Rec Ref: 11/LO/0396

IRAS 68898

Funder: The Wellcome Trust: WT092993MA

Version 4 - 22 February 2018

Date of Protocol: 22/02/18

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Study Summary

Title	A longitudinal study of alterations in metabolic markers and gut hormones in pregnant and non-pregnant patients with obstetric cholestasis and normal pregnant and non-pregnant controls.
Short Title	The Metabolic Profile in intrahepatic cholestasis of pregnancy and diabetes mellitus
Methodology	A prospective case control study

Study Duration	Until 30/04/2020
Objectives	To establish whether women affected by intrahepatic cholestasis of pregnancy (ICP)) and gestational diabetes mellitus (GDM) are predisposed towards developing metabolic disease in pregnancy and in later life. We will do this by measuring metabolic markers and assessing glucose homeostasis.
Population	Pregnant and non pregnant parous women
Eligibility	Pregnant and non pregnant women who have been affected by ICP and GDM together with matched controls

GLOSSARY OF ABBREVIATIONS

OC	Obstetric Cholestasis
ICP	Intrahepatic Cholestasis of Pregnancy
AE	Adverse Event
SAE	Serious Adverse Event
GCP	Good Clinical Practice
CI	Chief Investigator
GDM	Gestational Diabetes Mellitus

1. Background

CLINICAL FEATURES OF INTRAHEPATIC CHOLESTASIS OF PREGNANCY:

Intrahepatic cholestasis of pregnancy (ICP), is a pregnancy−specific liver disorder which typically presents with maternal pruritus (itching) in late pregnancy and affects about 0.7% of women in the UK. Biochemically, it is characterised by liver dysfunction with raised serum bile acids, and clinically by a significantly increased incidence of fetal complications, including spontaneous preterm labour, fetal distress, meconium staining of the amniotic fluid and sudden fetal death (Geenes and Williamson 2009).

The cause of ICP is complex and not fully understood. In addition the pathological mechanisms behind the adverse fetal outcomes have not been elucidated. The maternal disease is likely to be caused by interaction between sex hormone metabolites and bile acids in genetically susceptible women (Abu-Hayyeh et al. 2010). To date, we and others have identified genetic variation in several biliary transporters e.g. ABCB4, ATP8B1, ABCB11 and the main bile acid receptor, FXR, that predispose women to the disease (Dixon et al., 2000; Mullenbach et al., 2005; Pauli−Magnus et al., 2004; Van Mil et al, 2007; Dixon et al. 2009). Due to their inherent toxicity, bile acids are also likely to be responsible for the fetal component of the disease. Consistent with this the incidence of fetal complications has been shown to be increased in pregnancies where the levels exceed 40 uMol /L (Glantz et al., 2004). There are currently several theories about how bile acids may affect fetal wellbeing; with regard to the increased incidence of spontaneous prematurity, they have been shown to cause premature delivery in sheep (Campos et al., 1986), and increased myometrial contractility in response to oxytocin (Germain et al., 2003; Israel et al., 1986). Furthermore, bile acids are also known to cause increased colonic motility in rabbits (Snape et al., 1980) and cardiac dysrhythmias in rats (Williamson et al., 2001), which could explain meconium staining of the amniotic fluid and sudden fetal death respectively. While it is known that bile acids can be transported by proteins in the placenta, the precise role of the placenta in bile, lipid and glucose metabolism is not known. Our group has demonstrated that cholestasis alters lipid pathways in mouse placentas (unpublished data) but this has not been studied in humans.

BILE ACID HOMEOSTASIS:

Bile acids are the end product of hepatic cholesterol metabolism and act as the main route of excretion for cholesterol. In the adult human two primary bile acids are produced by the liver, i.e. cholic acid and chenodeoxycholic acid. There is some evidence that maternal bile acids gradually rise with advancing gestation, but they usually remain within normal limits (< 14 uMol /L) (Fulton et al., 1983; Pascual et al., 2002). This is in contrast to an ICP pregnancy, where maternal bile acids may be raised to 100 times the upper limit of normal (Walker et al.,2002). In OC, elevated levels of bile acids are also found in the fetal circulation (Laatikainen, 1975).

THE ROLE OF BILE ACIDS IN LIPID AND GLUCOSE METABOLISM:

There is accumulating evidence that FXR, the nuclear hormone receptor responsible for regulation of bile acid homeostasis, also plays a role in the regulation of lipid and glucose metabolism. FXR null mice have been shown to have abnormal blood lipid profiles including elevated plasma cholesterol, low density lipoprotein, high density lipoprotein and triglyceride levels (Ma, 2006). They also exhibit glucose intolerance and reduced insulin sensitivity (Zhang, 2006). Furthermore, ICP patients have raised levels of cholesterol and other lipid parameters (Dann et al. 2006). Specifically, the serum concentrations of low-density lipoprotein (LDL), apolipoprotein B-100, cholesterol and total cholesterol were markedly increased in women with ICP. They have also been shown to have impaired glucose tolerance (Wójcicka-Jagodzińska et al. 1989). Recent data from our group (Martineau et al. 2014, 2015) and others (Marschall et al. 2014) have shown that women with ICP have increased rates of GDM, and implicate raised serum bile acids in susceptibility to diabetes mellitus. Therefore this study will also include women with ICP and superimposed GDM in addition to women with GDM in the absence of ICP. This will enable us to evaluate the impact of cholestasis on the onset of diabetes mellitus in pregnant women.

In cholestasis bile acids accumulate in the liver, and this results in the induction of pathways that enhance bile acid excretion. This may also cause abnormal cholesterol and triglyceride levels. Bile acids also signal via FXR in the gut. Bile acids are stored in the gallbladder in the fasting state and released following ingestion of a meal. Once bile acids are in the intestine they are transported into enterocytes (gut cells) where they activate FXR which causes production and release of the hormone FGF19. FGF19 binds the FGF4 receptor on hepatocytes, and signals (via the jnk kinase pathway) to suppress cholesterol 7 alpha-hydroxylase (CYP7A1), a critical rate-limiting enzyme in synthesis of bile acids from cholesterol. There have been no studies of the gut-liver axis in pregnancy, but we hypothesise that abnormal bile acid signalling causes raised cholesterol in addition to increased serum bile acids.

Bile acids also bind the G-protein-coupled receptor (TGR5). In the gut this results in release of glucagon-like peptide-1 (GLP-1)(Thomas et al. 2009). GLP-1 is an anti-diabetic hormone that increases glucose-dependent insulin production and decreases glucagon production by the pancreas. To date, there have been no studies of the relationship between cholestasis and GDM and the levels of other fasting gut hormones that may influence glucose and lipid metabolism in pregnancy.

IMPORTANCE:

ICP is known to cause abnormal bile acid homeostasis and to be associated with an increased risk of diseases of the biliary system in later life. There have been small studies (Dann et al. 2006; Wójcicka-Jagodzińska et al. 1989) suggesting that it causes dyslipidaemia (raised lipids) and impaired glucose tolerance in pregnancy. However the underlying mechanisms of these abnormalities is not known. Similarly the influence of cholestasis on fetal metabolism is not known, and nor is the role of the placenta. It is also not known whether women with ICP have a predisposition to abnormal lipid and glucose homeostasis when they are not pregnant.

GDM is characterized by raised plasma glucose levels in pregnant women (in the absence of pre-pregnancy diabetes mellitus). This condition is associated with large-for-gestational age babies and obstructed labour. Women with GDM have increased risk of subsequent type 2 diabetes mellitus, and if they have this condition in a subsequent pregnancy there is an increased risk of stillbirth. This work is important to understand the causes of the metabolic abnormalities associated with ICP and GDM. If we demonstrate abnormal lipid and glucose profiles, these may be of relevance to the fetal complications of both disorders. It will also be of relevance to the future health of affected women and their children.

2. Hypothesis

There is a relationship between cholestasis and abnormalities of lipid and glucose homeostasis in the mother and baby

That there are abnormalities in bile acid, lipid and glucose homeostasis in women who have previously had ICP compared to controls.

That the placenta may play a role in bile, lipid and glucose homeostasis in ICP cases and controls

Some of the hormonal signals that cause ICP-associated GDM may also cause GDM in non-cholestatic women.

3. Study Objectives and outcomes

Objectives:

- To evaluate the relationship between bile acid, lipid and glucose metabolites in the mother and fetus in ICP, GDM and uncomplicated pregnancies.

Outcomes:

Primary Outcome

- To establish whether raised serum bile acids are associated with abnormalities in cholesterol and triglycerides in the mother and fetus

Secondary Outcome

- To establish the relationship between raised serum bile acids in the mother and fetus and abnormalities in:

1. Glucose homeostasis
2. Gut liver signaling hormones related to FGF 19 and C4 levels
3. Gut hormone secretion

4. Study design

In the non pregnant group we aim to recruit 40 women and 40 matched controls.
In the pregnant women with and without ICP or GDM we aim to recruit 40 in each group together with matched controls.
Written consent will be obtained from all participants.

Potential participants (both cases and controls) will be identified from the antenatal, obstetric medical and day assessment clinics at Queen Charlotte's and Chelsea Hospital, St Mary's Hospital and other participating units. Midwives and clinicians will be able to refer women who have previously had the conditions after asking them if they would be interested in speaking to someone from the research team regarding the study. Posters will be used in clinical areas and in the cafe area by the antenatal clinic to highlight the research. Women will also be approached directly in these clinics by researchers.

Non-pregnant females may be identified through organisations such as ICP Support and from posters in gynaecology clinics and hospital areas such as the entrance lobby to the main hospital where the shop and cash machine is situated. Clinicians and nurses will

also be able to refer women to the research team after asking the patient if they would be interested in speaking to someone from the research team.

The study will be a case-control study. The pregnancy cohort will be recruited prospectively and will undergo longitudinal study. The non-pregnant participants will also be recruited prospectively but this will be a cross-sectional study.

The study will comprise of the following groups of women. In the groups that comprise pregnant women, maternal blood will be collected at delivery together with placental samples and cord blood (once delivery has taken place and the cord has been clamped and cut). The collection of these types of samples will allow the researchers to fully investigate the metabolites, bile acids and hormones implicated in the disorder together with the potential impact they may have on both mother and baby.

Group 1 are non-pregnant women who have either had normal pregnancies in the past or who have had at least one pregnancy affected by intrahepatic cholestasis of pregnancy (ICP) and/or gestational diabetes mellitus (GDM). This group should only have to attend the study for one day.

Group 2 are women who are identified in early pregnancy. They are either expected to go on to have normal pregnancies or are women with a previous history of ICP and/or GDM. These women may be asked to participate up to three times. This will mean they will need to attend for three separate days. However, this will be linked to their regular visits to the hospital.

Group 3 are women who have been diagnosed during their current pregnancy with ICP and/or GDM. These women may be asked to participate twice. This will mean they need to attend for two separate days. However, this will be linked to their regular visits to the hospital which is likely to be at least once a week.

All participants will be given a standard meal (designed by a dietician) to eat the evening before the study day. Vegetarian and allergy needs will have been taken into account. They will then come to Queen Charlotte's & Chelsea Hospital (or other participating centres) at 8 am the following morning. Each participant will be given a choice regarding the collection of the blood samples. They can have an intravenous cannula inserted or

they can be bled separately each time or have a mixture of both procedures. For example, the first few samples could be taken separately and then a cannula could be inserted for the remaining samples that need to be collected and which are close in timing to each other. The first sample that is collected as soon as they arrive is a fasting sample.

Participants in group 1, 2 and 3 will be involved for most of the day, arriving at 8am in the morning and leaving around 4pm in the afternoon. Following the collection of the fasted sample outlined above participants will be provided with a breakfast. At one and two hours after breakfast blood samples will be taken. Finally, participants will have a standardised lunch with venous blood samples taken 15 minutes before consumption and further blood samples taken at the time of consumption and 20, 60, 120, and 180 minutes post consumption.

In total we will take 9 samples of blood from women in groups 1, 2 and 3. This will constitute a total of no more than 120 mls (approximately 9 tablespoons) of blood being taken on the day of the study.
The protocol will be performed only once for women in group 1. It will be performed twice for women in group 2 (at 11-13 weeks and 28-40 weeks). Women in group 2 who go on to develop ICP and/or GDM again in the current pregnancy will be transferred to group 3 at the time of diagnosis. Hence, it is possible that the protocol may be performed four times (11-13 weeks, third trimester, at the time of diagnosis, and 1-4 weeks after starting treatment with ursodeoxycholic acid). In reality this is unlikely to happen and hasn't so far in the study but we would like to retain this option should a participant meet the criteria and be happy to take part four times. For women in group 3 the protocol will be performed twice (at diagnosis and 1-4 weeks after starting treatment with ursodeoxycholic acid). We will record if women are also taking metformin. All of this will be discussed carefully with the participants and all participants will be advised that participating just once will still give us valuable data.

5. Eligibility

Inclusion Criteria

Inclusion criteria:
Cases

- Women with intrahepatic cholestasis of pregnancy, defined as pruritus in pregnancy in association with raised serum bile acids and in the absence of an alternative cause.
- Women with gestational diabetes mellitus (GDM) according to the diagnostic criteria used at the referring centre
- Non-pregnant parous females with previous ICP or GDM.
- Women who are able to give consent.
- Women >16 and <70 years of age.

Controls

- Pregnant women not affected by ICP or GDM .
- Non-pregnant parous females with previous uncomplicated pregnancy.
- Women who are able to give consent.
- Women >16 and <70 years of age.

Exclusion Criteria

Exclusion Criteria:

- Males.
- Non-pregnant females with other medical disorders that can cause liver impairment, abnormal lipid or glucose metabolism in pregnancy, e.g. pre-eclampsia, acute fatty liver of pregnancy, pre-existing diabetes mellitus
- Pregnant females with a history of other medical disorders that can cause liver impairment, abnormal lipid or glucose metabolism in pregnancy, e.g. pre-eclampsia, acute fatty liver of pregnancy, pre-existing diabetes mellitus
- Women who are not able to give consent.
- Women <16 and >70 years of age.

6. Adverse Events

An **adverse event (AE)** is any untoward medical occurrence in a participant during the study participation days. An existing medical condition (unless it worsens on the study participation days) is not classed as an AE.

All AEs will be recorded in the notes of the participant and on an AE form

A **serious advent event (SAE)** is any untoward and unexpected medical occurrence or effect that:

- Results in death
- Is life threatening to mother and/or baby
- Requires hospitalization
- Results in persistent or significant disorder incapacity ability

All SAEs and action taken as a result will be recorded in the notes of the participant and on an SAE form. An SAE form should be completed and faxed to the Chief Investigator within 24 hours. All SAEs should be reported to the appropriate research ethics committee where in the opinion of the Chief Investigator, the event was:

- 'related' i.e. resulted for the administration of any research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event using the NRES SAE form for non-IMP studies.
Contact details for reporting SAEs
Professor Catherine Williamson – 0207 594 2197/07971 193686
Fax: 0207 594 2154 Attention: URGENT - Professor Catherine Williamson
Please send SAE forms to Professor Catherine Williamson, Dept of Surgery & Cancer, IRDB Building, Du Cane Road, London, W12 ONN

It is not anticipated that there will be any increased risk of adverse events or serious adverse events in this study. However, it could be that a participant may have an unexpected reaction to the food they eat i.e. an allergic reaction and appropriate action will need to be taken in the event of this happening.

It is also possible that a participant may experience a vasovagul (fainting) episode during venepuncture and appropriate care of the participant will implemented should this happen.

7. Statistics

- Data analysis will take place at the Institute of Reproductive and Developmental Biology, Imperial College, London. It will be undertaken by the research scientists with statistical advice from the divisional statistician.
- We will use conventional statistical analysis including tests of normal distribution and either Students T-test or the Mann-Whitney U test as appropriate.

8. Procedure for entry to the study, schedule for sample analysis and data recording

- The study will obtain ethics approval from NHS Research and Ethics Committee and Trust R&D prior to starting recruitment.
- Patient information sheets will be provided and informed consent obtained.
- Participants will be asked to give consent to have their medical records examined by members of the research group. This will be undertaken in order to check any previous data that maybe of relevance to the study e.g. previous history of ICP or GDM
- The personal addresses telephone numbers and possibly e−mail addresses of participants are kept on file by the research group for future reference. This information is stored in a locked filing cabinet in a room within the research centre that is locked when not in use. Access is only granted to members of the research group.
- The personal data of the participants is also stored on a university computer. The computer is non net−worked and it is password protected. Access is only available to members of the research group
- Samples are linked-anonymised when they come into the laboratory for processing. The codes are kept in the PI's room (Professor Williamson) and only members of the research team have access to the coding book.
- Samples may be made available to collaborators, but these will be linked-anonymised and written consent from the participant will be obtained. If participants do not want their samples to be made available to collaborators they will have the option to opt out of this.
- Personal data is stored in a locked filing cabinet in a different building within a locked filing cabinet in the research centre. Any data relating to results are also coded and retained on a non net worked computer.

- Only members of Professor Williamson's research team who are involved with the study will have access to the personal information included in the questionnaire. They will also have access to the medical records of the participants but only with the signed consent of the participants.
- Relevant data such as blood results may also be made available to collaborators, but this will be in a linked anonymised format, and written consent from the participant will be obtained.

9. Regulatory Issues

9.1. Ethical Approval

Prior to starting the study, we will seek ethical approval from NHS Research Ethics Committee (NHS REC). Amendments to the protocol will be made with the approval of the chief / principal investigator(s) and will be subject to review by the REC. The study will be carried out in accordance with the Declaration of Helsinki.

9.2. Consent

For participants recruited at Queen Charlotte's and Chelsea Hospital and St Mary's Hospital a member of the research team will meet with the participant to explain the study to them and to answer any questions that they may have. They will have as much time as they need to think about the study and will be given an information sheet to take home to look at, together with a letter explaining what happens next should they wish to take part, a medical questionnaire (which they will need to complete to check eligibility) and a pre-paid envelope. Following this, the participant will be given an appointment to come in to hospital to be formally recruited to the study and sign a consent form

Participants recruited via the patient organisation will be sent an information sheet about the study via email/post. This gives them time to consider whether they are interested in taking part. If they are, they will be sent a letter acknowledging their interest and giving them the opportunity to ask any questions about the research. The letter will also include a consent form and a medical questionnaire. If they need to discuss the study they will be contacted at a time to suit them. Following this they will be asked to sign the consent form and return it to us in the post together with the completed questionnaire.

In addition to a written information sheet, all women will be provided with contact details for members of the research group and will be encouraged to make contact should they have any questions or concerns about the study. It will be made explicit to the participants that they can change their mind about taking part at any stage of the recruitment process and during the research period itself.

9.3. Confidentiality

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the date Protection Act

9.4. Indemnity

Imperial College holds negligent harm and non-negligent harm insurance policies which apply to this study.

9.5. Sponsor Imperial College London will act as the main sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

9.6 Trust R&D Any amendments should also be approved by the sponsor and Trust R&D department.

9.7. Funding

The study has been funded by The Wellcome Trust and is also supported through internal funding.

Participants will be awarded (in vouchers) £20 for every study day they take part in, up to a potential maximum of £60.

9.8. Audits and Inspections

The study is subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the UK Policy framework for health and social care.

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