

Mental health and asthma control during pregnancy: Investigating underlying immune mechanisms

Isabella-Rose Sibly Meredith

BSc, B Health Science (Hons)

Submitted in fulfilment of the requirements for the degree of
M. Philosophy (Medical Science)

School of Paediatrics and Reproductive Health,
Discipline of Obstetrics and Gynaecology,
University of Adelaide

February 2015

Supervisors: Associate Professor Vicki Clifton, Dr Luke Grzeskowiak,
Dr Annette Osei-Kumah

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Isabella-Rose Meredith

Date

Table of Contents

List of Figures	6
List of Tables	7
Acknowledgements.....	8
Abbreviations	9
Abstract.....	12
Chapter 1: Literature Review.....	13
1.1. Asthma and Exacerbations.....	13
1.2. Asthma and Pregnancy	15
1.3. Mental Health and Asthma	15
1.4. Immune Mechanisms of Asthma and Pregnancy	16
1.4.1. Immune Cells and Asthma.....	16
1.4.1.1. T lymphocytes	17
1.4.1.2. Monocytes.....	20
1.4.2. Inflammation Subtypes in Asthma.....	23
1.4.2.1. Eosinophilic Inflammation.....	23
1.4.2.2. Neutrophilic Inflammation.....	25
1.4.3. Lung Function in Pregnancies Complicated by Asthma.....	27
1.5. Immune Mechanisms of Mental Health.....	28
1.5.1. Asthma and Mental Health.....	30
1.5.2. Anxiety	30
1.5.3. Depression.....	32
1.5.4. Dopamine and Serotonin	32
1.5.5. Asthma, Pregnancy and Mental Health.....	33
1.6. Conclusion.....	37
1.7. Research	37
1.7.1. Knowledge Gap	37
1.7.2. Research Questions.....	38
1.7.3. Hypotheses	38
1.7.4. Aims	38
Chapter 2: Methodology.....	40
2.1. Part A: Subject Recruitment and Assessment	40
2.1.1. Subjects.....	40
2.1.2. Maternal Data Collection.....	41
2.1.3. Assessment of Maternal Asthma	42
2.1.4. Assessment of Maternal Depression/Anxiety.....	42
2.1.5. Sample Collection	42

2.2.	Part B: Epidemiology	43
2.2.1.	Statistics.....	43
2.3.	Part C: Immune Cell Experiments.....	44
2.3.1.	FACS Analysis of Cell Surface Molecules.....	44
2.3.1.1.	Staining of Cell Surface Molecules	44
2.3.1.2.	Flow Cytometry Analysis	45
2.3.1.3.	Statistics	45
2.3.2.	Chemotaxis	46
2.3.2.1.	Optimisation.....	46
2.3.2.1.1.	Cell number and Incubation Time.....	46
2.3.2.1.2.	nfMLP vs. MCP-1	46
2.3.2.1.3.	FBS Concentration	47
2.3.2.2.	Final Protocol.....	47
2.3.2.3.	Statistics	48
Chapter 3:	Results - Epidemiology.....	49
3.1.	Part A: Maternal Demographics.....	49
3.2.	Part B: Epidemiology	50
3.2.1.	Asthma Exacerbations	51
3.2.2.	Uncontrolled asthma	54
3.3.	Discussion	56
Chapter 4:	Results – Immune Cell Experiments.....	59
4.1.	Part A: Flow cytometric analysis of cell surface molecules (FACS).....	59
4.1.1.	Maternal Demographics	59
4.1.2.	Percentage of Total Monocytes.....	60
4.1.3.	CD14Bright, CD16Bright, CD14 ⁺ CD16 ⁺ and CD14 ⁺ CD16 ⁻ Monocytes	62
4.1.4.	Adhesion Receptor and HLA-DR Expression	62
4.1.5.	Effect of Uncontrolled Asthma and Asthma Exacerbations	63
4.2.	Part B: PBMC Chemotaxis.....	65
4.2.1.	Maternal Demographics	65
4.2.2.	PBMC Chemotaxis	67
4.3.	Discussion	67
4.3.1.	FACS Analysis.....	67
4.3.2.	PBMC Chemotaxis	69
Chapter 5:	Discussion	71
5.1.	Discussion	71
5.2.	Strengths and Limitations	73
5.3.	Further work.....	75
5.4.	Conclusion	76

References.....	77
Appendix	85

List of Figures

Figure 1: Factors influencing asthma control.....	14
Figure 2: The interactions of the major immune cells involved in the pathophysiology of asthma	17
Figure 3: The interactions of inflammatory cells and cytokines involved in asthma, depression/anxiety and pregnancy.....	36
Figure 4: Visiting schedule of participants and experimental immune cell analysis.....	41
Figure 5: The FlowJo gating strategy for sorting total monocytes from Tc cells (A) and distinguishing CD14 Bright, CD16 Bright and intermediate (CD14 Bright + CD16 Bright) monocytes from each other.....	45
Figure 6: Diagram of chemotaxis assay using a Transwell® insert.....	47
Figure 7: No effect of depression/anxiety in pregnancies complicated by asthma on the percentage of women experiencing an exacerbation throughout gestation (A; p=0.362). Frequency of exacerbations was also unchanged with the presence of depression/anxiety in pregnancies complicated by asthma (B; p=0.589).....	53
Figure 8: The presence of depression/anxiety in pregnancies complicated by asthma significantly increased the percentage of women experiencing uncontrolled asthma throughout gestation (A; p=0.017). The number of uncontrolled asthma events was also significantly increased with the presence of depression/anxiety in pregnancies complicated by asthma (B; p=0.009).....	55
Figure 9: Percentage total monocytes in the peripheral blood mononuclear cells of pregnant women with and without asthma and with and without depression/anxiety at 18 (A) and 30 weeks gestation (B).....	61
Figure 10: Percentage CD11a expression on CD14 Bright monocytes in pregnant women with and without asthma and with and without depression/anxiety during pregnancy at 30 weeks gestation.....	63
Figure 11: Effect of uncontrolled asthma on percentage total monocytes at 18 weeks gestation.....	64

List of Tables

Table 1: Experimental numbers at 18 weeks and 30 weeks gestation for each group for the flow cytometric analysis of cell surface molecules (FACS) performed on monocytes.....	44
Table 2: Experimental numbers at 18 and 30 weeks gestation for each group for the chemotaxis assays performed on the peripheral blood mononuclear cells (PBMCs).....	47
Table 3: Participant data at booking visit (12 or 18 weeks gestation).....	49
Table 4: Participant data of asthmatic women at booking visit (12 or 18 weeks gestation).....	51
Table 5: Asthma control data of women throughout pregnancy.....	52
Table 6: Monocyte flow cytometric analysis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy.....	59
Table 7: Percentages of total, CD14Bright, CD16Bright, CD14+CD16+ and CD14-CD16- monocytes in pregnant women with and without asthma and with and without depression/anxiety at 18 and 30 weeks gestation.....	62
Table 8: Peripheral blood mononuclear cell chemotaxis subgroup data at booking visit (12 or 18 weeks gestation) and asthma control throughout pregnancy.....	65
Table 9: Migration index of peripheral blood mononuclear cells throughout pregnancy.....	67

Acknowledgements

I would like to acknowledge the support of my supervisors Vicki Clifton, Luke Grzeskowiak and Annette Osei-Kumah. Vicki, you have been a great support and inspiration over the past two years, thanks for all the time and energy you put into me, I could not have done this without you. Luke thanks for your help and willingness to supervise me late in the day. Your insights and expertise has given me a have really enhanced my research experience. Annette, your help in the laboratory has been crucial to my development as a researcher and so I want to take this opportunity to thank you for all your help and support.

Also to my family and friends who supported, encouraged and calmed me down when things were crazy. I could not have completed this without you all.

Abbreviations

ACQ: Asthma Control Questionnaire

ACTH: Adrenocorticotrophic hormone

ANRQ: Antenatal Risk Questionnaire

AQLQ: Juniper Asthma Quality of Life Questionnaire

AVP: Vasopressin

BMI: Body mass index

CD: Cluster of differentiation i.e. CD14

CD14Bright: 'Classical' monocytes

CD16Bright: 'non-classical' monocytes

CD14⁺CD16⁺: 'Intermediate monocytes

CCR2: Monocyte chemoattractant protein-1 receptor

CeA: Central nuclei of the amygdala

CI: Confidence interval

CRH: Corticotrophin-releasing hormone

DC: Dendritic cell

DPBS: Dulbecco's phosphate buffered saline

EGF: Epidermal growth factor

EMT: Epithelial-mesenchymal transition

EPDS: Edinburgh Postnatal Depression Score

FACS: Flow cytometric analysis of cell surface molecules

FBS: Foetal bovine serum

FCV: Force vital capacity

FENO: Fractional exhaled nitric oxide

FEV1: Forced expiratory volume in one second

GR: Glucocorticoid receptor

HADS: Hospital Anxiety Depression Scale

HLA: Human leukocyte antigen

HPA axis: Hypothalamic-pituitary-adrenal axis

ICAM: Intercellular Adhesion Molecule

ICS: Inhaled corticosteroids

IFN: Interferon

IgE: Immunoglobulin E

IL: Interleukin

IQR: Interquartile range

IRR: Incidence rate ratio

LABA: Long acting β 2 agonists

LMH: Lyell McEwin Hospital

LPS: Lipopolysaccharide

M1: Classically activated macrophage

M2: Alternatively activated macrophage

MCP: Monocyte chemoattractant protein

MeA: Medial nuclei of the amygdala

nfMLP: N-formly-met-leu-phe

NK: Natural killer cells

OCS: Oral corticosteroids

OVA: Ovalbumin

PBMC: Peripheral blood mononuclear cell

RANTES: Regulated And Normal T cell Expressed and Secreted

RR: Relative risk

SGA: Small for gestational age

Tc cells: T cytotoxic cells

TGF: Transforming growth factor

Th cells: T helper cells

TNF: Tumor Necrosis Factor

T reg: Regulatory T cells

VEGF: Vascular endothelial growth factor

Abstract

Background: Asthma during pregnancy has been associated with poor pregnancy outcomes such as pre-eclampsia, small for gestational age babies and preterm birth. Depression and anxiety are associated with reduced asthma control in non-pregnant individuals. This study investigated whether depression/anxiety in combination with pregnancies complicated by asthma has a negative effect on asthma control. Potential immune mechanisms that may drive worsening asthma were also investigated.

Methods: One hundred and eighty-nine asthmatic women with and without depression/anxiety were followed throughout their pregnancies. Incidences of uncontrolled asthma and exacerbations were measured throughout gestation. At 18 and 30 weeks of gestation, monocyte inflammatory profile was examined using flow cytometric analysis of cell surface molecules (FACS) and peripheral blood mononuclear cell (PBMC) chemotaxis was also examined.

Results: The incidence of uncontrolled asthma increased in women with depression/anxiety compared to women without depression/anxiety during pregnancy (unadjusted incidence rate ratio (IRR) 1.739, adjusted IRR 1.633, CI 1.092-2.442, $p=0.017$). Relative risk of experiencing uncontrolled asthma during pregnancy was also increased with depression/anxiety (unadjusted RR 1.619; adjusted RR 1.538, CI 1.114-2.122, $p=0.009$). There was no increase in the incidence rate ratio (unadjusted IRR 0.770; adjusted IRR 0.755, CI 0.412-1.382, $p=0.362$) or relative risk (unadjusted RR 0.867; adjusted RR 0.859, CI 0.496-1.489, $p=0.589$) of asthma exacerbations during pregnancies complicated by depression/anxiety. Asthma without depression/anxiety was associated with an increase in peripheral blood total monocyte percentage at 18 but not 30 weeks gestation when compared to asthmatic women with depression/anxiety ($p=0.027$). There were no changes in PBMC chemotaxis at 18 or 30 weeks gestation in pregnant women regardless of the presence of asthma or depression/anxiety.

Conclusion: The presence of asthma and depression/anxiety during pregnancy is associated with an increase in uncontrolled asthma, but not a change in exacerbation risk. This increase in uncontrolled asthma in women with depression/anxiety was not a result of alterations in monocyte inflammatory profile or PBMC chemotaxis.