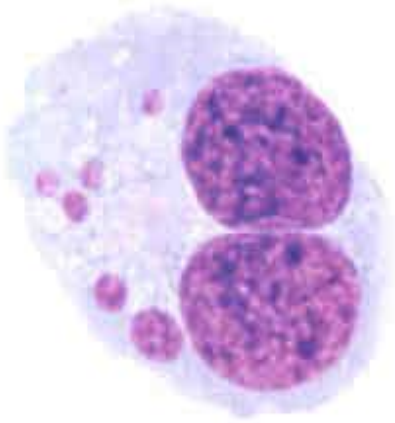

Diet and DNA damage in infants
The DADHI study

Mansi Dass Singh

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The DADHI study

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MSc (Nutrition & Dietetics)

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And

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Genome Health and Personalised Nutrition

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This thesis is dedicated to my guide and father Mr Harikishan Dass

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Abstract

Accumulation of DNA damage during infancy may increase risk of accelerated ageing and degenerative diseases such as cancers. Pregnancy is understood to be a state of high expression of inflammatory genes. It may be possible that infants, born to women at high risk of pre-eclampsia (PE): a condition associated with increased oxidative stress, inflammation and altered gene expression, may have increased DNA damage compared with infants born to women at low risk of developing PE. However, currently there are no baseline DNA damage data for infants born to mothers in relation to their low/high risk of developing PE in Australia.

This PhD project had four phases:

***A systematic literature search** was conducted with the aim to explore the literature and identify knowledge gaps in the role of folate in the etiology and prevention of PE. The review found (i) deficiency of folate and other B vitamins, with higher concentrations of oxidative stress biomarkers in maternal tissues and body fluids of women with PE when compared with women at low risk of PE, and (ii) some of this dysregulation may be balanced epigenetically with oral intake of methyl donors including folate and vitamins $B_{2.}$

***A prospective cohort study** was conducted; 'Diet and DNA damage in Infants' (The DADHI study), with the aim to study:

(i) DNA damage, cytostasis, and cytotoxicity utilizing a comprehensive Cytokinesis block micronucleus cytome (CBMN-Cyt) assay in lymphocyte of Australian born infants [at birth (cord blood, n=82), 3 (n=64) and 6 months (n=53) (heel prick blood)] of mothers at low risk of PE

(ii) association of maternal factors and infant birth outcomes with CBMN-Cyt biomarkers

(iii) whether mode of feeding influences CBMN-Cyt biomarkers in infants at 3 and 6 months after birth

This study found significant positive associations of infant birth outcomes (gestation age, birth weight, head circumference, birth length and APGAR score) and maternal anthropometric variables with CBMN-Cyt biomarkers, suggesting possible genotoxic effects on infant's DNA by metabolic processes that promote excessive growth and higher body mass index.

* The next aim was to determine

(i) association of **blood micronutrient status** with CBMN-Cyt biomarkers in cord blood at birth and infant's blood at 3 and 6 months

(ii) whether mode of feeding influences blood micronutrient status at 3 and 6 months after birth

The study observed significant associations of DNA damage biomarkers with infant birth outcomes and micronutrient status suggesting that both under and oversufficiency of some nutrients may be detrimental for cell growth and repair.

*A **pilot project** [in 'Investigations in the Folic acid clinical trial' (INFACT study)] with the aim to collect DNA damage data in the cord blood collected from infants of women at increased risk of developing PE. The study found that (i) maternal anthropometric variables may influence infant birth outcomes, mainly birth size, and (ii) INFACT cases (n=10) had higher frequency of CBMN-Cyt biomarkers compared with gender and birth weight matched DADHI controls (n=15).

These preliminary data could be used to form the design of larger studies required to confirm the association of maternal factors and PE with DNA damage in the infants at birth and later in life in the first 1000 days.

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.

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Mansi Dass Singh (----------2017)

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And last but above all, my Father who has been the inspiration, initiator and motivating luminous for my dreams and aspirations and mom for her endearing and blessings.

Abbreviations

8-OHdG: 8-hydroxy-2′- deoxyguanosine
5-methyl THF: 5 methyl tetrahydro folate
5-LTR: 5-long terminal repeat

AOAC: Association of official analytical methods
ATP: Adenosine triphosphate
ADP: Adenosine diphosphate
ATM: Ataxia-telangiectasia mutated
ANOVA: Analysis of variance

BNC: Binucleated lymphocyte cells
BMI: Body mass index
BF: Breast fed
BP: Blood pressure

CBMN-Cyt: Cytokinesis block micronucleus-cytome assay
CO_2: Carbon dioxide
CH3: methyl group
Cob: Cobalamin
Cfu: Colony forming units
CVD: Cardiovascular disease
CI: Confidence interval
Cyto-B: Cytochalasin-B
CpG: cytosine-phosphate-guanine
CSIRO: Commonwealth Scientific and Industrial Research Organisation
CV: Coefficient of variation
CB: Calibration blank
CIROS: circular optical systems
COBRA: combined bisulfate restriction analysis
COMT: catechol-*O*-methyltransferase
CRH: corticotropin-releasing hormone
CT: cytotrophoblasts

DADHI: Diet and DNA damage in Infants
DHF: Di hydrofolate
DNA: Deoxyribonucleic acid
d-ROM: derivatives of reactive oxygen metabolites

dUMP: deoxy uridine monophosphate
dTMP: deoxy thymidine monophosphate
dTTP: deoxy thymidine triphosphate
dUMP: deoxy uridine monophosphate
DMSO: Dimethylsulphoxide
DS: Down syndrome

EDTA: Ethylene diamine tetra acetic acid
ELISA: Enzyme-linked immunosorbent assay
FA: Folic acid
FFQ: Food frequency questionnaire
FBS: Foetal Bovine serum
FAn: Fanconi Anemia
FACT: Folic Acid Clinical Trial
GA: Gestation age
HELLP: haemolysis, elevated liver enzymes, low platelet count
HIF-1α: hypoxia induced factor-1α
Hcy: Homocysteine
HBSS: Hanks Balanced Salt solution
HPLC: High Performance Liquid Chromatography
HT: Hypertension
IUGR: Intrauterine growth restriction
IGF: Insulin growth factor
IMVS: Institute of Medical and Veterinary Science
IRR: Incident rate ratio
IVF: In vitro fertilization
ICP: Inductively coupled plasma analysis
ICPAES: Inductively coupled plasma atomic emission spectrometry
IQ: Intelligence quotient
INFACT: Investigations in Folic Acid Clinical trial
ICAM-1: intercellular adhesion molecule-1
ICR: imprinting control region

L *casei: Lactobacillus casei*
LBW: Low birth weight
LGA: Large for gestational age
LOD: Limit of detection

MTHF: Methyl tetrahydro folate
MTHFD1: methylenetetrahydrofolate dehydrogenase
MTHFR: methylenetetrahydrofolate reductase
MTRR: methionine synthase reductase
MTR: methionine synthase
MN: Micronuclei
MNC: Mononucleated lymphocyte cells
MMA: Methylmalonic acid
MDA: malondialdehyde
MS: Microsoft

MA: Microbiological assay
MRL: method reporting limits
MMP: matrix metalloproteinase
MS-SNuPE: methylation-sensitive single-nucleotide primer extension

NHANES: National Health and Nutrition Examination Survey
NHMRC: National Health and Medical Research Council's levels of evidence
NPB: Nucleoplasmic bridges
NBUD: Nuclear buds
NDI: Nuclear division index
NTD: Neural tube defects
NSW: New South Wales

OR: Odd ratio
OCM: One carbon metabolism
OSI: oxidative stress index

PE: Pre-eclampsia
PCR: Polymerase chain reaction
p: significance value
PHA: Phytohemagglutinin
PABA: Para amino benzoic acid
PBL: Peripheral blood lymphocyte
PTPE: preterm pre-eclampsia

RCT: randomized controlled trial
RBC: Red blood cells
RCF: red cell folate
r: correlation coefficient
RR: relative risk
RNA: Ribonucleic acid
ref-1: redox factor
RT-PCR, reverse transcription polymerase chain reaction

SD: standard deviation
SEM: standard error of mean
SAM: S-adenosylmethionine
SAH: S-adenosyl homocysteine
SGA: Small for gestation age
SSE: sister chromatin exchange
THF: tetra hydro folate
TNF: Tumor necrosis factor
TLR-9: toll like receptor-9
TS: thymidylate synthase
TAS: total antioxidant status
TOS: and total oxidant status
WCH: Women's and Children Hospital

Publications arising from this thesis

1. Singh MD, Thomas P, Owens J, Hague W, Fenech M, 2005. 'Potential role of folate in Pre-eclampsia', Nutrition Reviews .Oct; 73 (10):694-722. Impact factor 6
2. Singh MD, Thomas P, Hor M, Almond T, Owens J, Hague W, Fenech M 2016. 'Infant birth outcomes are associated with DNA damage biomarkers as measured by CBMN-Cyt assay-The DADHI study'. Submitted with major revisions to Mutagenesis journal

Presentations arising from this thesis

1. 'Genome stability of infants as measured by CBMN-Cyt assay and influence of feeding during six months after birth' at Nutrition society of Australia-Adelaide Student presentation event, 19 November 2015
2. 8th Congress of the International Society of Nutrigenetics/Nutrigenomics 2-3 May 2014, Gold Coast, Australia
3. Florey postgraduate Research Conference, 24th September, 2015
4. Joint Annual Scientific Meeting of the Nutrition Society of NZ and the Nutrition Society of Australia, 1st - 4th December 2015
5. 'Genome stability in lymphocytes of South Australian babies as measured by Cytokinesis Block Micronucleus assay', Oral presentation as part of Annual review at joint HDR seminar programme for the Disciplines of Obstetrics and Gynaecology and Robinson Institute, 12th March 2015
6. Folate and Genome Integrity in Infants', Oral presentation as part of Annual review at joint HDR seminar programme for the Disciplines of Obstetrics and Gynaecology and Robinson Institute, 10th June 2014
7. Diet and DNA Health in Infant', Oral presentation at CSIRO Nutrigenomic Laboratory, June 2014