THE ASSOCIATION BETWEEN INSULIN RESISTANCE AND EARLY SPONTANEOUS MISCARRIAGES IN JOS - PLATEAU STATE

A DISSERTATION SUBMITTED TO THE NATIONAL POSTGRADUATE MEDICAL COLLEGE OF NIGERIA IN PART FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE FELLOWSHIP OF THE COLLEGE FACULTY OF OBSTETRICS AND GYNAECOLOGY

BY

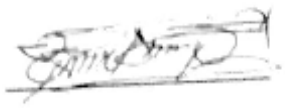
DR. ADIKPE EMMANUEL EDUGBE

(MBBS UNI-JOS, 2008)

MAY 2018

DECLARATION

I **Dr Adikpe Emmanuel Edugbe** hereby declared that this work is original. The work has not been submitted in support of an application for a fellowship/degree/diploma of this or any other institution of learning. It has also not been submitted for publication/conference presentation.



Signature

25th July 2017

Date

JOS UNIVERSITY TEACHING HOSPITAL
JOS, NIGERIA

Phone: 073-450226-9
E-mail: juth@infoweb.abs.net

Cables & Telegram: JUTH
P.M.B. 2076
JOS

Ref:.....

Date: 19th January, 2018

CERTIFICATION

This dissertation title" **THE ASSOCIATION BETWEEN INSULIN RESISTANCE AND EARLY SPONTANEOUS MISCARRIAGES IN JOS PLATEAU STATE** " *Was* carried out by **Dr. A.E. EDUGBE** under the supervision of the signed Consultants in the Department of Obstetrics and Gynaecology of the Jos University Teaching Hospital (JUTH).

Sign.....
Date.....

Dr. A. N Ocheke
Head of Department
Obstetrics and Gynaecology

SUPERVISORS PAGE

The study documented in this dissertation was performed by **Dr Adikpe Emmanuel Edugbe** under our supervision. We also supervised the writing of the dissertation.

Name of Training institution - Jos University Teaching Hospital, Jos Plateau State.

Name of Supervisor**: Dr T. Oyebode, MBBS, FWACS, FMCOG**

Status of Supervisor: Consultant Obstetrician and Gynaecology, Jos University Teaching Hospital.

Signature..... Date 26/7/2017

Name of Supervisor**: Prof P.H. Daru, MBBS, FWACS**

Status of Supervisor: Consultant Obstetrician and Gynaecology, Jos University Teaching Hospital.

Signature..... Date 26/07/17

Name of Supervisor: **Dr L.C. Imoh, MBBS, FMCPath, Dip. Toxicology (Cardiff)**

Status of Supervisor: Consultant Chemical Pathologist, Jos University Teaching Hospital

Signature..... Date 24/7/2017

v

JOS UNIVERSITY TEACHING HOSPITAL
JOS, NIGERIA

Phone: 073-450226-9
E-mail: juth@infoweb.abs.net

Cables & Telegram: JUTH
P.M.B. 2076
JOS

Ref: JUTH/DCS/ADM/127/XIX/ 6274

Date: 31st August, 2015.

Dr. Adikpe Emmanuel Edugbe,
Department of Obstetrics & Gynaecology,
Jos University Teaching Hospital,
Jos-Nigeria.

RE: ETHICAL CLEARANCE/APPROVAL

I am directed to refer to your application dated 30th August, 2015 on the research proposal titled:

"The Association between Insulin Resistance and Early Spontaneous Miscarriages in Jos, Plateau State"

Following recommendation from the Institutional Health Research Ethics Committee, I am to inform you that Management has given approval for you to proceed on your research topic as indicated.

You are however required to obtain a separate approval for use of patients and facilities from the department(s) you intend to use for your research.

The Principal Investigator is required to send a progress report to the Ethical Committee at the expiration of three (3) months after ethical clearance to enable the Committee carry out its oversight function.

Submission of final research work should be made to the Institutional Health Research Ethical Committee through the **Secretary, Administration Department,** please.

On behalf of the Management of this Hospital, I wish you a successful research outing.

Comfort A. Onoja
For. Chairman, MAC

INSTITUTIONAL HEALTH RESEARCH ETHICAL COMMITTEE
DESPATCHED
31 AUG 2015

PLATEAU STATE SPECIALIST HOSPITAL JOS

Ref. No. PSSH/ADM/ETH. CO/2015/016
Old Bukuru Road,
P.M.B. 2113,
Jos, Nigeria
Tel. 073-462180
Fax: 073-464031

Email: psshjos@yahoo.com

Date: November 15, 2017

Reg. No: NHREC/05/01/2010b
NOTICE OF EXPEDITED REVIEW AND APPROVAL

Re: The Association between Insulin Resistance and Early Spontaneous Miscarriages in Jos Plateau State

Name of Principal Investigator: Dr. Adikpe Emmanuel Edugbe
Address of Principal Investigator: Jos University Teaching Hospital, Dept. of Obstetrics and Gynaecology
Date of receipt of valid application: November 14, 2017.
Date of meeting when final determination of research was made: November 15, 2017.

This is to inform you that the research described in the submitted protocol, has been reviewed and *given expedited approval by the Health Research Ethics Committee.*

This approval dates from **15/11/2017** to **15/11/2018**. Note that no participant accrual or activity related to this research may be conducted outside of these dates. You may liaise with the Hospital record department for necessary cooperation / assistance.

All informed consent forms used in this study must carry the HREC assigned number and duration of HREC approval of the study. In multiyear research, endeavor to submit annual report to the HREC early in order to obtain renewal of your approval and avoid disruption of research. *The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rides and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the HREC. No changes are permitted in the research without prior approval by the HREC except in circumstances outlined in the Code. The HREC reserves the right to conduct compliance visit your research site without previous notification.*

Dr. Bitrus Matawal, MBBS, FWACS
Chairman, HREC PSSH

FERTILE GROUND HOSPITAL

Plot 1234 Zaramaganda, Jos. Adjacent Golden Bread.
E-mail: dregbodo@yahoo.com | Tel: 08035977944

13th November, 2017

Dr Adikpe Emmanuel EDUGBE,

Department of Obstetrics & Gynaecology,

Jos University Teaching Hospital (JUTH),

Jos- Plateau State.

RE: ETHICAL CLEARANCE

This is to inform you that your application for ethical clearance dated 8th November, 2017 on the research titled:

"The Association between Insulin Resistance and Early Spontaneous Miscarriages in Jos Plateau State" has been reviewed and given approval by the Ethical Committee of the Hospital.

You are expected to comply with all institutional guidelines and ethical principles.

You are required to submit a copy of the completed research to the ethical committee through the hospital secretary.

On behalf of the management of the hospital, I wish you a successful research outing.

Dr Christopher .O. Egbodo, MBBS, FWACS, MPH

Medical Director/Chairman Ethical Committee

Dr. Christopher O. Egbodo MBBS, FWACS (CEO) Pharm. (Mrs) Egweye Egbodo B.Pharm, FPCPharm Jeremiah Egbodo

DEDICATION

This study is dedicated to Almighty God, the fountain of all knowledge and wisdom, and to all women with first trimester recurrent spontaneous miscarriages.

ACKNOWLEDGEMENT

I am most grateful to God Almighty for giving me the grace and enablement to complete this dissertation.

I appreciate the contributions and encouragement of all the consultants in the department of obstetrics and gynaecology for consistently showing interest in my training and progress. I am very grateful to Dr. T. Oyebode, my lead supervisor of this dissertation for her untiring support and guidance towards the success of it. I want to thank Prof P.H Daru who is also my supervisor for providing the necessary encouragement and conducive atmosphere for carrying out this work.

May I use this opportunity to express my profound gratitude to Dr L. Imoh of the department of Chemical pathology JUTH and his wife, Mrs. Joy Imoh for painstakingly helping me in the study from the time of development of the proposal to the data analysis and final write up.

My heart goes out to Dr Egbodo, Dr Musa, Dr Ocheke, Dr Ekwempu, Dr Pam I.C, Dr Nyango, Dr Kahansim, Dr Pam VC, Dr Anyaka, Dr Magaji, Dr Madziga, Dr Shambe and Dr Karshima, for being there for me. Special thanks to Prof Sagay, Prof Ujah (mni) and Prof Mutihir for their fatherly roles.

My appreciation also goes to all the nurses in the department of Obstetrics and Gynaecology, especially those in the antenatal clinic their support to the success of this work.

To my fellow colleagues in the department, both seniors and juniors, I say thank you for your love, care and support. I am grateful.

Finally I would like to thank my beautiful angel, Mrs Deborah Edugbe for her encouragement, patience and care. To Ohelume, Onahinyohe and Ahinyohe, thanks for your sacrifices and understanding.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AUCG	Area under the Curve of Glucose
AUCI	Area under the Curve of Insulin
BMI	Body Mass Index
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosteronesulphate
ELISA	Enzyme Linked Immunosorbent Assay
FI	Fasting Insulin
FG	Fasting Glucose
FPG	Fasting Plasma Glucose
GA	Gestational Age
g	Gram
GLUT-1	Glucose Transporter 1
≥	Greater than or equal
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
H_0	Null Hypothesis
H_1	Alternate Hypothesis
ICSI	Intra-cytoplasmic Sperm Injection
IGFBP-1	Insulin-like Growth Factor Binding Protein 1
IL	Interleukin
IR	Insulin Resistance
ISIcomp	Composite Insulin Sensitivity Index
IVF	In vitro Fertilisation

JUTH	Jos University Teaching Hospital
Kg	Kilogram
≤	Less than or equals to
LH	Luteinising Hormone
m	Metre
ml	Millilitre
µ	Microlitre
mmol/L	Millimoles per Litre
OGTT	Oral Glucose Tolerance Tests
OR	Odd Ratio
PAI-1	Plasminogen Activator Inhibitor 1
PAI-2	Plasminogen Activator Inhibitor 2
PCOS	Polycystic Ovary Syndrome
pmol/L	Pico moles per litre
±	Plus or Minus
QUICKI	Quantitative Insulin sensitivity Check Index
RSM	Recurrent Spontaneous Miscarriage
SM	Spontaneous Miscarriage
SPSS	Statistical Package for the Social Science

ABSTRACT

BACKGROUND: Recurrent spontaneous miscarriages (RSM) remain a major challenge to gynaecologists as 50% of aetiologies of spontaneous miscarriages are unknown. Insulin resistance (IR) has been implicated as an aetiological factor. However, there are insufficient and conflicting evidence regarding the contribution of insulin resistance to the occurrence of RSM.

OBJECTIVE: To investigate the relationship between insulin resistance and first trimester recurrent spontaneous miscarriages in comparison with first trimester normal pregnancies.

DESIGN: Case control study.

METHODOLOGY: This was a case-control study involving 80 women with first trimester RSM (cases) and 80 Women with normal first trimester pregnancies with at least one successful pregnancy and no history of miscarriages (Controls). From each participant fasting blood glucose (FG) was assayed by automated colorimetric enzymatic analysis and fasting insulin (FI) was assayed by BIOS Human insulin ELISA kits. Data was analysed using SPSS version 22.0.

RESULTS: There were no significant differences between the mean age and BMI of cases and controls ($p= 0.990$ and 0.930 respectively). FG was significantly higher in cases compared to controls, (4.77±1.14 vs 3.58±0.78)mmol/L, $p<0.001$, however no significant difference in FI between cases and controls, 26.62±14.62 vs 25.15±13.61mIU/L, $p= 0.509$. Prevalence of IR (HOMA-IR >4.5) and (FG/FI ratio <4.5) for cases and controls are 48.8% vs 27.5% and 63.8% vs 53.8% respectively. HOMA-IR >4.5 was statistically significant between cases and controls, $p = 0.009$.

CONCLUSION: This study suggests that women with recurrent first trimester miscarriages are more likely to be insulin resistant with higher fasting blood glucose levels compared to women with normal first trimester pregnancies.

KEY WORDS: First trimester recurrent miscarriages, Insulin resistance, HOMA-IR

CHAPTER ONE

1.1 INTRODUCTION

Spontaneous miscarriages (SM) remain a frustrating experience to the couples and their families, especially when it is recurrent. Considering the fact that, the exact cause(s) of recurrent early pregnancy loss is not known in over 50% of cases, this has become a topic of consideration by researchers. It affects 1% of all women.[1,2,3] The incidence of spontaneous miscarriages may be much greater than is clinically recognized.[1] Spontaneous miscarriages occur in 12 to 15% of all pregnancies. [1,3,4] Recurrent spontaneous miscarriages (RSM) affect 2-4% of reproductive age couples, representing a challenge for the attending clinicians.[5] It affects both naturally conceived pregnancies and those obtained after assisted reproductive technology treatment.[6]

A broad spectrum of factors have been described in the aetiology of RSM, these include chromosomal anomalies, genetic, hormonal, anatomic, systemic, immunologic, infectious and endocrine but 50% of cases remain unexplained.[1,4,7,8] Endocrine disorders have been frequently linked to recurrent pregnancy loss. Because embryo attachment and early implantation are exquisitely controlled by the local hormonal milieu, endocrine-related pregnancy failures are likely to occur early in gestation.[2] Endocrine causes include poorly controlled diabetes, polycystic ovary syndrome (PCOS), hyperandrogenism, luteal phase defect and thyroid disorders. Insulin resistance (IR), hyperinsulinaemia and hyperandrogenaemia are claimed to be a potential causes of the high rate of pregnancy loss in patients with PCOS and

have been linked with the metabolic and endocrine abnormalities associated with pathophysiology of recurrent spontaneous miscarriages. [2,7,9-10]

Recent studies showed that insulin resistance (IR) can be a cause, independent of PCOS status.[1,5,6,11] Women with insulin resistance are at an increased risk of insulin resistance during the first trimester of a new pregnancy.[5,6,11] Recent meta-analysis concluded that insulin resistance is associated with the susceptibility to recurrent miscarriages.[12] Therefore, insulin resistance might be one of the direct causes of recurrent miscarriages.[1, 13]

A meta-analysis of seven clinical trials of association between recurrent miscarriages and insulin resistance showed that the fasting insulin level and homeostasis model assessment-insulin resistance index (HOMA-IR) were higher and fasting blood glucose insulin ratio was lower in the study groups than that of the control groups.[12]

Insulin resistance has been demonstrated to increase expression of plasminogen activator inhibitor-1 (PAI-1),[6,14] an endogenous inhibitor of fibrinolysis.[5,15,16] PAI-1 activity is known to elevate the level of serum insulin and it induces a hypofibrinolytic state.[14,17] This creates a thrombotic milieu at the maternal-foetal interface with a high risk of miscarriage.[15,16,18,19] Insulin resistance is also known to play a critical role in the ovarian androgen excess and therefore might promote miscarriage by increasing circulating androgen concentration.[5,20,21]

Hyperinsulinaemia has been shown to decrease the expression of glycodelin and insulin like growth factor binding protein-1 (IGFBP-1) at the implantation site.[6,21-23]

Glycodelin plays an important role in inhibiting the endometrial response towards the embryo, while IGFBP-1 facilitates the adhesion process of the blastocyst at the foeto-maternal interface.[7,24,25] Studies have also suggested that insulin resistance and or hyperinsulinemia may have deleterious metabolic effects, including causing an increase in plasma level of homocysteinemia.[7,25] Hyperhomocysteinaemia may impair pregnancy by interfering with endometrial blood flow and vascular integrity;[17,26] it increases the oxidative stress in vascular endothelium, activates the platelet and has been documented to increase the probability of early pregnancy loss.[7,27] The polycystic ovary syndrome like status, due to elevated fasting insulin (FI) and high IR, and the successful use of metformin for the treatment of PCO suggested the idea that metformin could be used in these women.[7,28,29]

Metformin use in PCOS women with insulin resistance demonstrated various beneficial effects: a decrease in the level of PAI-1, enhancement of the uterine vascularity, and alleviation of the IR, insulin levels, and HOMA-IR.[7] Insulin resistance has further been associated with abnormal endometrial development and endometrial defects. [2,7]Palombo et al[1,5,22] reported that lowering the insulin levels with metformin enhances uterine vascularity and reduces uterine vascular resistance; after metformin[7] use the vascular resistance in spiral arteries was reduced with 20%.

Metformin is a biguanide, an insulin sensitizer which reduces plasma glucose concentrations in Type 2 diabetes patients.[28,30] Metformin can induce weight loss in some patients thereby reducing IR.[21,30] Metformin predominantly works by reducing

hepatic glucose production, inhibiting gluconeogenesis both directly and indirectly (by decreasing free fatty acid concentrations).[28,29,30] Data suggest that it improves peripheral insulin sensitivity.[30] Studies with metformin in PCOS revealed reductions in androgen levels.[15,22,28] Metformin exerts systemic and local effects in reducing miscarriage risk.[22,31] It reduces insulin and PAI-1 levels, plasmatic endothelin-I, androgens, and LH concentrations and by increasing serum IGFBG-1 levels and glycodelin levels.[15,22,31] It exerts its local effects on the endometrium, and embryonic factors. On the uterus, metformin improves several surrogate markers of endometrial receptivity, endometrial and sub-endometrial vascularization and uterine artery blood flow. Similarly, metformin normalizes the ovarian artery impedance, and peri–corpus luteum vascularization.[22]

1.2 JUSTIFICATION FOR THE STUDY

Recurrent spontaneous miscarriages can be frustrating to couples. Unfortunately, spontaneous miscarriages are the most common complication of pregnancies. Recurrent spontaneous miscarriages (RSM) remain a major challenge to the gynaecologists as 50% of aetiologies of RSM cannot be explained. Intensive research efforts are still in progress to unravel the possible aetiological factors associated with this rather difficult problem so as to contribute positively towards its effective management. Insulin resistance is one of the treatable factors that have been implicated as a possible direct cause of RSM in recent studies. However, there are insufficient and conflicting evidence (association and no association) in the literature regarding the role of insulin resistance in miscarriages. Therefore, there is a need to evaluate the relationship between insulin resistance and first trimester recurrent miscarriages in order to contribute to the existing body of knowledge and

effective prevention. This research also will shed light on the contribution of insulin resistance in the occurrence of first trimester recurrent pregnancy losses in Jos, North central Nigeria.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Spontaneous Miscarriage

The definition of spontaneous miscarriage has continued to vary from place to place as a result of differences in the age of foetal viability. A spontaneous miscarriage is defined as the spontaneous loss of pregnancy before a foetus reaches viability.[32] This include all pregnancy losses up to the 24th week of gestation.[32] However in our environment, miscarriage is the termination of pregnancy before 28 weeks of gestation.[33] First - trimester miscarriage occurs before 12 weeks' gestation and accounts for the majority.[34] The overall rate is 20%. Early pregnancy loss is defined as the termination of pregnancy before 20 weeks of gestation or with a foetal weight of < 500g.[35] Ectopic and molar pregnancies are not usually included in this definition. Recurrent miscarriage is classically defined as three or more consecutive pregnancy losses at 20 weeks' gestation or less or with foetal weights less than 500g; however testing the women after 2 losses could spare them of another pregnancy failure; thus the definition was modified lowering the number of spontaneous losses to two.[1,20,36,37]

2.2 Risk factors for Spontaneous Miscarriage

In addition to insulin resistance, the other causes[1,3,32,36,38] of pregnancy losses includes advanced maternal age which is associated with chromosomal anomalies, genetic factors (most spontaneous miscarriages are caused by an abnormal (aneuploid) karyotype of the embryo, at least 50% of all first-trimester spontaneous miscarriages are cytogenetically abnormal), previous history of pregnancy loss, endocrine factors (poorly controlled diabetes mellitus, polycystic ovarian syndrome,

thyroid antibodies, luteal phase deficiency), haematological factors (antiphospholipid syndrome, factor V Leiden, prothrombin gene mutations, protein C, protein S, and antithrombin III deficiencies, hyperhomocystienaemia, methyltetrahydrofolate reductase enzyme mutation), lifestyle (cigarette smoking has been suggested to have an adverse effect on trophoblastic function and is linked to an increased risk of sporadic pregnancy loss, cocaine use, alcohol consumption 3 to 5 drinks per week, and increased caffeine consumption >3 cups of coffee, have been associated with risk of miscarriage), infections (ureaplasmaurealyticum, mycoplasma hominus, chlamydia, listeria monocytogenes, toxoplasma gondii, rubella, cytomegalovirus, herpes virus, and other less frequent pathogens have been identified more frequently in vaginal and cervical cultures and serum from women with sporadic miscarriages), immunologic factors (human leucocyte incompatibility/absence of maternal antibodies, uterine natural killer cells. cytokines), anatomical factors (uterine anomalies, cervical anomalies, leiomyomas, intrauterine adhesions).

2.3 Insulin Resistance and Recurrent Spontaneous Miscarriages

Insulin resistance is a condition in which the efficacy of insulin in promoting the absorption and utilization of glucose by organs, tissues, and cells is lower than normal.[11] Individuals with IR show glucose levels that are either normal or high, and insulin levels that are more or less than normal.[11] The association between insulin resistance and pregnancy loss is difficult to explain and scanty materials exist regarding the independent effect of IR on spontaneous miscarriages. However, Insulin resistance (IR) has been reported to play an important role in recurrent spontaneous pregnancy loss (RSPL) among patients with polycystic ovary syndrome (PCOS).[6,13,39]

Previous studies have shown that using metformin may reduce the risk of pregnancy loss in PCOS women,[14] and IR is a baseline predictor of clinical efficacy of metformin use for improving fertility performance.[29] Given the well-known effect of metformin in reducing IR, these results have indirectly suggested that IR was correlated with the risk of spontaneous miscarriages.

In a Romanian study,[7] recurrent pregnancy loss was associated with high insulin resistance evidenced by significantly higher fasting insulin level and HOMA-IR in the case group than control group; 15.24±3.5μU/mL versus 12.83±3.2μU/mL, $P<$ 0.001 and 2.98 versus 1.69, $P<$ 0.05 respectively. The result of the study supports the idea that insulin resistance may be involved in the aetiology of recurrent pregnancy loss. The significantly higher value of FI and HOMA-IR in the case group might suggest that not only the IR but also hyperinsulinaemia may have an adverse effect on pregnancy outcome.

A study[6] on the effect of IR on spontaneous miscarriages in a Chinese population who conceived via assisted reproductive technology treatment (IVF and ICSI), it was found that the pregnancy rate was 32.7% (107 of 327), the mean age was 30.8yrs, ranging from 21–39yrs, and the mean BMI was 22 kg/m^2, ranging from just over 16 to nearly 33 kg/m^2, the mean IR was 3.28, with over 20% (23 of 107) patients who were insulin resistant. There were no cases of diabetes in this group of patients. The overall spontaneous abortion rate was 17.8% (19 of 107). The risk of spontaneous abortion by factors that was included in the logistic regression model. For women aged 35yrs or older, the risk is almost doubled, 26.9 vs. 14.8% in younger women.

Overweight/obesity, compared with normal weight, was also linked with nearly doubling the risk of spontaneous abortion. PCOS was linked with a significant increase of the risk. However, none of the associations reached a statistically significant level as shown by the OR calculated from the logistic regression analysis. Patients with IR greater than 4.5 had significantly greater risk of spontaneous abortion compared with those with normal insulin resistance-index. This increase was significant as shown by multivariate logistic regression analysis (OR, 8.32; 95% CI, 2.65–26.13). Another logistic regression analysis, using HOMA-IR as a continuous variable and the same other variables, showed an increase in OR of 0.52 (95% CI, 0.34–0.80) per unit HOMA-IR. This study suggested that IR was an independent risk factor for spontaneous miscarriage. The association of IR with the risk of spontaneous pregnancy loss was significant after adjusting for other risk factors.

Another study in China[11] give credence to the association between IR and recurrent spontaneous miscarriages in women with non-PCOS status. There were no statistically significant differences found in the FG, FI, HOMA-IR, and HOMA-β between the patient and control groups. However, 1, 2, and 3-hour plasma glucose and insulin levels and the AUCG and AUCI of the patient group were higher than those of the control group. The calculated ISIcomp was lower for the patient group than the control group. These differences were statistically significant. This suggests that IR occurs in non-PCOS patients with recurrent miscarriages. To buttress this relationship 6 women in the patient group had PCOS. In order to eliminate their contribution to the positive results, the data was re-analysed after excluding these 6 women. The exclusion of the PCOS did not influence the indices of IR suggesting

that a history of recurrent miscarriages is related to IR and not necessarily to PCOS status.

2.4 Possible Pathophysiology of the Association between Insulin Resistance and Recurrent Spontaneous Miscarriages

The mechanism by which insulin resistance and compensatory hyperinsulinemia contribute to miscarriage is not clearly understood,[11,24] but may involve decreased expression of endometrial glycodelin and IGFBP-1 production, resulting in an inhospitable endometrial milieu.[6,21-24]Glycodelin and IGF binding protein-1 (IGFBP-1) are major endometrial secretory proteins that may play significant roles in endometrial receptivity in early pregnancy and in the maintenance of pregnancy.[25]Glycodelin is a glycoprotein produced by decidualized endometrial glands during the luteal phase, and facilitates implantation by inhibiting the immune response of the endometrium to the embryo. Decreased serum concentrations of glycodelin are associated with retarded endometrial development, early pregnancy loss, and recurrent miscarriage. IGFBP-1 is a protein that facilitates adhesion processes at the foeto-maternal interface during the peri-implantation period.[7,22,23] In non-pregnant women, IGFBP-1 is produced primarily by the liver and is negatively regulated by insulin. During pregnancy, IGFBP-1 is produced by the endometrium as well.

One hypothesis is that IR causes an uncontrolled diabetic-like state in the foetal environment resulting in increased first trimester pregnancy losses.[5] High insulin levels have been shown in vitro to increase the transport of glucose by first trimester

cytotrophoblasts independent of glucose level (probably by up regulation of the GLUT1 glucose transporter system).[5]

Another possibility involves plasminogen activator inhibitor-1 (PAI-1), an endogenous inhibitor of fibrinolysis which has been demonstrated to increase with IR and increasing levels of insulin and decrease with treatment with metformin, an insulin-sensitizing agent.[5,16] Gris et al.[5] found high levels of PA inhibitor activity in women with recurrent pregnancy losses of unknown origin. It has been suggested that increased PAI-1 activity promoted recurrent abortions through thrombotic induction of placental insufficiency.[5]

Insulin resistance is known to play a critical role in ovarian steroidogenesis due to a compensatory insulin secretion.[20,21] Increased insulin concentrations cause hyperandrogenism.[21] Insulin directly promotes ovarian androgen steroidogenesis, and inhibits liver release of the sex hormone binding globulin (SHBG) and production of insulin-like growth factor binding protein 1 (IGFBP-1).[21] Interestingly, concentrations of sulfated DHEA (DHEAS) are also increased in the blood.[30] DHEAS is secreted exclusively by the adrenal glands. The mechanism of increased DHEAS production by the adrenals is not yet known, although insulin and IGF-1 have been shown to up regulate adrenal 17-hydroxylase and 17, 20-lyase activity.[28] Cocksedge et al.[20] showed that hyperandrogenaemia is associated with increased risk of a further miscarriages in women with previous miscarriages. This finding gives credence to the findings of previous studies which investigated the prevalence

androgen excess in women with recurrent miscarriages using measurement of either testosterone or free androgen index.[20]

Studies also affirmed that insulin resistance and or hyperinsulinaemia causes increase in the plasma level of homocysteine.[25] Elevated levels of homocysteine interfere with vascular integrity and endometrial blood flow thus leading to pregnancy failure.[17,26] Insulin-resistant PCOS have elevated levels of homocysteine, and this could be responsible in part for the decreased implantation rates and increased miscarriage rates of patients with PCOS, even with effective induction of ovulation or IVF.[40]

2.5 The Effects of insulin Sensitizers on Insulin Resistant Women with Spontaneous Miscarriages

It is no longer in doubt the positive roles insulin sensitizers play in reducing the miscarriage rate in women with insulin resistant PCOS and IR independent of PCOS. In the last decade researchers have undertaken several trials to prove the efficacy of insulin sensitizers; biguanides and thiazolidinediones in improving many aspects of the multifactorial PCOS and IR.[30]

Trials have been done with metformin, troglitazone and rosiglitazone. Metformin belongs to the class of biguanides and acts by decreasing the tissue resistance to insulin through increased glucose uptake in skeletal muscle and fatty tissue, decreasing hepatic gluconeogenesis, and decreasing intestinal glucose uptake.[21,30] In addition, metformin therapy decreases androgen concentrations, increases SHBG release, and induces spontaneous ovulation and pregnancy in some patients.

Metformin therapy improves insulin sensitivity in patients with diagnosed insulin resistance.

Reduction in the incidence of pregnancy loss was noted among PCOS women after treatment with metformin, presumably due to its effect on IR[6,14]. Insulin sensitization effect of metformin has been associated with an increase glycodelin and IGFBP-1 levels and uterine vascularity during the luteal phase, a decrease in PAI-1 activity especially in women who had a favourable pregnancy outcome, and a reduced androgen level.[18,41] Nonrandomized studies have shown that the reduction in insulin levels with metformin in insulin resistant individuals may reduce miscarriage risk by restoring normal haemostasis and improving the endometrial milieu.[14,42] It was also reported that metformin has beneficial metabolic, endocrine, vascular, and anti-inflammatory effects on the risk factors contributing to early pregnancy loss.[31] Significant reduction in the rate of early pregnancy loss in pregnant women with PCOS was documented as well as tolerance with a minimum of side effects to metformin.[24]

Troglitazone and rosiglitazone represent a novel class of drugs; Thiazolidinediones (TZDs) that decrease peripheral insulin resistance by enhancing insulin action in the skeletal muscle, liver and adipose tissue.[30] Studies with thiazolindinediones in PCOS subjects have shown an improvement of the androgen levels and ovulation rate and enhanced insulin sensitivity without any reduction in the weight of subjects.[28]Troglitazone was however, withdrawn from the market in 2000 due to hepatotoxicity. Studies have now been done with Rosiglitazone showing a decrease

in testosterone, androstenedione and DHEA levels and an increase in SHBG (thereby causing a decrease in free testosterone levels) along with an improvement in insulin sensitivity.[28]

2.6 Assessment of Insulin Resistance

There are several methods of assessing insulin resistance and sensitivity. These include hyperinsulinaemic-euglycaemic clamp, fasting glucose, fasting insulin, fasting glucose/fasting insulin ratio, oral glucose tolerance test (OGTT), homeostasis model assessment-insulin resistance index (HOMA-IR), composite insulin sensitivity index (matsuda index), Belfiore glycaemic index, Belfiore free fatty acid glycaemic index, McAuley index, quantitative insulin sensitivity check index, Bergman's minimal model etc.[43,44]

There is however, no universally accepted single adequate marker of insulin resistance that would be both highly sensitive and specific. The hyperinsulinaemic-euglycaemic clamp technique is regarded as the gold standard worldwide.[5,17,21] It provides highly reproducibly steady-state estimates of insulin-mediated glucose clearance in peripheral tissues.[43] This technique is however, laborious, expensive and time-consuming and thus more applicable to research than to daily clinical practice.[5,21,43]

Fasting insulin and glucose concentrations are easy markers to obtain. Fasting insulin values equal to 20 mIU/L or higher indicate the presence of insulin resistance while fasting glucose to fasting insulin ratio less than 4.5 is regarded as characteristic for insulin resistance.[5,17,21] FG/FI ratio has been identified as a quite sensitive and specific marker of insulin resistance.[5,21]

Another method, based on measurements of fasting glucose and insulin levels, is the homeostatic model assessment (HOMA-IR). This method has been reported to correlate well with the clamp technique and thus provides quite a good assessment of insulin resistance in clinical practice.[17,44] It was first described by Matthews DR, et al, in 1985. It describes a method for assessing insulin resistance/insulin sensitivity and β-cell function (an index for insulin secretion) from fasting glucose and insulin or C-peptide concentrations. The original model (HOMA1) calculates the insulin resistance and B cell function from the following equations.[43,45,46]

(1) HOMA1-IR = (FPI x FPG)/22.5 for Insulin Resistance

(2) HOMA1-%β = (20 x FPI)/(FPG - 3.5) for β-cell function

Where FPI is fasting plasma insulin concentration (mIUl/L) and FPG is fasting plasma glucose (mmol/L). Insulin resistance is defined by HOMA-IR index >4.5.[5,6,17] Resistance to insulin however, was diagnosed at HOMA-IR index ≥3.8 in another study.[21] Generally there is no universally accepted score to define IR[47,48] , HOMA-IR ≥75th percentile of the normal population defines insulin resistance in that population or 25th percentile with the least insulin sensitivity in a given population defines IR in that population.[48,49]

Computer model (HOMA2) which takes into consideration the specificity of the insulin assay used as well as making allowance for a wider range of insulin secretions is gradually replacing HOMA1 in practice. The HOMA Index has gained wide acceptance as simpler tool for assessing insulin resistance compared to the gold-standard hyperinsulinaemic-euglycaemic insulin clamp method.[19,45] The HOMA model however, as an index of insulin sensitivity, measures predominantly hepatic type insulin sensitivity utilizing fasting plasma glucose and fasting plasma insulin.[45-47]

CHAPTER THREE

OBJECTIVES OF THE STUDY

3.1 GENERAL OBJECTIVE

The aim of this study was to investigate the relationship between insulin resistance and first trimester recurrent spontaneous miscarriages in comparison with first trimester normal pregnancies.

3.2 SPECIFIC OBJECTIVES

i. To determine the prevalence of insulin resistance among women with first trimester recurrent spontaneous miscarriages and women with normal first trimester pregnancies.

ii. To determine the association between first trimester recurrent spontaneous miscarriages and insulin resistance.

3.3 NULL HYPOTHESIS

There is no association between insulin resistance and first trimester recurrent spontaneous miscarriages.

3.4 ALTERNATE HYPOTHESIS

There is an association between insulin resistance and first trimester recurrent spontaneous miscarriages.

CHAPTER FOUR

MATERIALS AND METHODS

4.1 STUDY AREAS

The study was conducted in the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital (PSSH) and Fertile Ground Hospital (FGH), all of which are located in the cosmopolitan city of Jos, Plateau State, North Central Nigeria and provide Obstetric and Gynaecological services.

The Jos University Teaching Hospital is a 600 bed capacity tertiary centre. It receives referrals from neighbouring states of Nassarawa, Bauchi, Benue, Gombe, Adamawa, Taraba and Kaduna. It has a well-established department of Obstetrics and Gynaecology.

Plateau State Specialist Hospital (PSSH) is 250 bed capacity tertiary health institution that provides specialised medical services, trains health professional and serves as research centre. It has a functional gynaecological and maternity units. It receives referrals from all the general hospitals in the 17 local government areas of the state and neighbouring states.

Fertile Ground Hospital (FGH) on the other hand, is a privately owned 33 bed capacity specialist hospital which in addition, provides assisted reproductive technology (ART) services.

4.2 STUDY DESIGN

This was a case control study.

4.3 STUDY POPULATION

The study population comprised of all consenting women with history of recurrent first trimester miscarriages seen at the gynaecological clinics and women presenting with recurrent first trimester miscarriages at the gynaecological emergency units of JUTH, PSSH and FGH. The control group comprised of consenting first trimester pregnant women who presented at the emergency rooms with reasons other than miscarriages or booking antennal care clinics. Pregnancy was defined based on ultrasound documentation of intrauterine gestation or presence of normal trophoblastic tissues on histopathological examination. First trimester pregnancy loss was defined as any spontaneous miscarriage occurring on or before 13 weeks of gestation. Gestational age was determined by ultrasound dating.

4.4 DATA COLLECTION

Eighty cases and controls each were recruited for the study from August 2016 to January 2018 by consecutive sampling technique. Women with recurrent first trimester miscarriages were recruited into the case group, while the control group comprised of women within the same age range with at least one successful pregnancy and no history of miscarriages, presenting for routine antenatal care or at the emergency room for reasons not related to miscarriage at ≤ 13 weeks of gestation. Consent was obtained from the respondents before data collection.

A structured questionnaire was administered and privacy was ensured while interview was being conducted. Serial numbers were assigned to each patient in both groups to protect her identity and eliminate bias in laboratory assessment. The

chemical pathologist was blinded to this number coding used to identify the patients so as to eliminate bias. Five (5) millilitres of blood was collected from the cubital fossa of each participant into a plain vacutainer tube for fasting blood glucose (FG) and fasting insulin (FI) determination. The blood samples were centrifuged at 5000rpm for 5 minutes after clotting and the sera collected were stored at -20^{0}C until the required sample size was reached after which they were analysed. The information obtained included; age, parity, ethnicity, gestational age, weight, height, body mass index, occupation and number of consecutive miscarriages.

4.5. LABORATORY TESTS

For the purpose of this study, fasting blood glucose and fasting insulin were assayed and HOMA-IR and FG/FI ratio were calculated. Insulin resistance (IR) was defined as HOMA-IR >4.5 and FG/FI ratio <4.5.[5,6,16,19] The assays were done by a chemical pathologist according to the kit manufacturer's specifications. Fasting blood glucose was analysed by automated colorimetric enzymatic analysis using commercial kits on the Cobas c111 automatic analyser (COBAS Roche Diagnostic, D-68305 Mannheim, Germany and DRG Diagnostics). Fasting insulin was assayed by BIOS Human insulin ELISA kits (Chemux Bioscience, Inc. USA).

4.6 PROCEDURE

The collected sera were allowed to thaw at room temperature and then analysed for fasting blood glucose and fasting insulin by securing 20 micro-titre wells in a frame holder. 25µL each of Standard, control and samples were dispensed with new disposable tips into appropriate wells. As part of quality control measures some

samples were duplicated. 25µL of Enzyme Conjugate was dispensed into each well and thoroughly mixed for 10 seconds.

The mixture was incubated for 30 minutes at room temperature, after which the content of the wells were shaken out briskly. The wells were rinsed 3 times with diluted Wash Solution (400µL per well) and then Stricken sharply on absorbent paper to remove residual droplets. 50µL of Enzyme Complex was added to each well and then incubated for 30 minutes at room temperature. The content of the wells were briskly shaken out and then rinsed 3 times with diluted wash Solution (400µL per well) after which the wells were stricken sharply on absorbent paper to remove residual droplets. 50µL of Substrate Solution was then added to each well and incubated for 15 minutes at room temperature.

The enzymatic reaction was stopped by adding 50µL of Stop Solution to each well. The absorbance (OD) of each well was determined at 450±10 nm with a micro-titre plate reader, read within 10 minutes after adding the Stop Solution. A calibration curve was then generated for each batch of assays.

The reference range of the method is 2-25mIU/L and the analytical measuring range is 1.76-100.0mIU/L. The method has no significant interference with bilirubin up to 50mg/dl and haemoglobin up to 400mg/dl and triglyceride up to 300mg/dl.

Calculation of Results

A calibration curve was plotted using absorbance obtained from each standard against its concentration. The absorbance value was plotted on the vertical (Y) axis and concentration on the horizontal (X) axis. The corresponding concentration of the sample was obtained from the calibration curve.

Quality Control

The analytical accuracy and precision was assured by simultaneous analyses of pooled serum quality control specimen with each batch of samples. The auto-analyser was calibrated and the routine maintenance was done in line with the manufacturer's specifications. The inter-batch CV for glucose and Insulin was 1.0 %. The Intra and Inter-batch CV for Insulin were 1.9% and 9.0% and these are within recommended limits.

4.7 INCLUSION CRITERIA FOR CASES

Women who presented with first trimester recurrent miscarriages or history of at least 2 consecutive first trimester miscarriages who had evaluation for other causes of miscarriage.

4.8 INCLUSION CRITERION FOR CONTROLS

Women with normal first trimester gestations with at least one successful pregnancy and no history of miscarriages.

4.9 EXCLUSION CRITERIA CASES

1. Women who refuse to give consent.
2. Women with previous pregnancy losses attributable to other causes.
3. Women who are known diabetics.
4. Women with other forms of gestations (ectopic, molar).
5. Women with diagnosis of PCOS. PCOS status was determined based on the revised Rotterdam criteria: (1) Oligo- and/or anovulation, (2) clinical and/or biochemical signs of hyperandrogenism, and (3) polycystic ovaries with exclusion of other aetiologies (congenital adrenal hyperplasia, androgen

secreting tumours, or Cushing's syndrome). The diagnosis was made if any 2 out of 3 criteria were met.

4.10 EXCLUSION CRITERIA FOR CONTROLS

1. Women who refuse to give consent.
2. First trimester pregnant women with a history of miscarriage.
3. Known diabetics or women with history of gestational diabetes.
4. Women with first trimester pregnancies with prior diagnosis of PCOS (as above).

4.11 ESTIMATION OF SAMPLE SIZE

The sample size was estimated using the formula;

$$n = \frac{\{P1\ (1-P1) + P2\ (1-P2)\}\ X\ (Z\ \alpha + Z\ \beta)^2}{(P1-P2)^2}$$

Where: n: number of sample size in each of the group.

P1= proportion of insulin resistance among cases (0.24 in a similar study).[16]

P2= proportion of insulin resistance among controls (0.08 in the same study).[16]

Z-α/2= value of standard normal distribution corresponding to a significance level of alpha (1.96 for two-sided test at the 0.05)

Z-β/2=value of standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%)

$$n = \frac{\{(0.24\times0.76 + 0.08 \times 0.92)\}\ x\ (1.96 + 0.84)^2}{(0.16)^2}$$

$n \approx 78$

The sample size was adjusted to 80 for the cases and 80 for the controls to compensate for an attrition rate of 5%.

4.12 DATA ANALYSIS

All statistical analyses were performed using SPSS software (version 22.0). Frequencies and percentages were computed for demographic characteristics of the cases and controls. Student's t-test, Chi- square test or Fisher's exact test were used to test the difference between groups where appropriate. A p-value of <0.05 was considered as statistically significant at confidence interval (Cl) of 95%.

4.13 ETHICAL CONSIDERATION

Ethical clearances were obtained from the Ethical Committees of the Jos University Teaching Hospital (JUTH), Plateau State Specialist Hospital (PSSH) and Fertile Ground Hospital (FGH). The nature, aim and objectives of the study were explained to each woman and consent obtained before recruitment into the study. The women were offered the option to opt out of the study, bearing in mind such action would not in any way compromise the quality of care they would receive at any service point in hospitals.

Appropriate instruments were used for blood sample collection to prevent harm to the patients. The specimens obtained were used for the research purpose only. All information gotten was strictly confidential and patients' identity was not disclosed. Results were explained to the patients and no information was withheld.

The women comprising the case and control groups had a free fasting blood glucose, fasting insulin and insulin resistant status determined. Those identified with abnormal results were counselled accordingly and offered appropriate treatment.

CHAPTER FIVE

5.1 RESULTS

This study consisted of 160 participants; 80 women with first trimester recurrent spontaneous miscarriages (cases) and 80 women with first trimester normal pregnancy (controls). The demographic characteristics of women that participated in the study are as shown in table 1. The cases and control groups were comparable in terms of maternal and gestational ages. The mean age of women that comprised the cases was 28.09±6.14 years and that of the control group was 28.10±6.21 years.

The mean gestational age of the study participants was 10.68±1.52 weeks and 11.19±1.87 weeks for cases and controls respectively with an overall mean gestational age of 10.94±1.70 weeks for both groups. The mean parity was 0.61±0.68 and 1.58±0.92 for the cases and the controls respectively with a statistically significant difference, $p<0.001$. The overall mean parity of the study population was 1.07±0.80. The mean number of miscarriages in the case group was 2.55±0.83. Sixty percent of the study population had tertiary education, 2.5% had no formal education and 30.0% were housewives. There were no significant differences with respect to levels of education, ethnicity and occupation between the cases and controls, $p > 0.05$. The three major Nigerian ethnic groups constituted 20.6% and 24.4% of cases and controls respectively.

Table 1: Demographic characteristics of the study participants.

Characteristics	Cases N = 80(%)	Controls N = 80(%)	Total N = 160(%)	p-value
Age (Yrs.)				
Mean ± SD	28.09 ± 6.14	28.10 ± 6.21	28.10±6.18	0.990
Parity				
Mean ± SD	0.61 ± 0.68	1.58 ± 0.92	1.07±0.80	<0.001†
GA (Weeks)				
Mean ± SD	10.68 ± 1.52	11.19 ± 1.87	10.94±1.70	0.066
No. miscarriages				
Mean ± SD	2.55±0.83	0.00±0.00	1.28±0.42	<0.001†
Ethnicity				
Hausa	19(23.8)	23(28.7)	42(26.2)	
Igbo	7(8.7)	7(8.7)	14(8.8)	0.792*
Yoruba	7(8.7)	9(11.3)	16(10.0)	
Others	47(58.8)	41(51.3)	88(55.0)	
Education				
No formal	4(5.0)	0(0.0)	4(2.5)	
Primary	5(6.3)	4(5.0)	9(5.6)	0.164**
Secondary	22(27.5)	29(36.3)	51(31.9)	
Tertiary	49(61.2)	47(58.7)	96(60.0)	
Occupation				
House wife	21(26.3)	27(33.8)	48(30.0)	
Self-employed	25(31.2)	25(31.2)	50(31.3)	
Student	8(10.0)	9(11.3)	17(10.6)	0.789**
Teaching	6(7.5)	3(3.7)	9(5.6)	
Banking	2(2.5)	1(1.3)	3(1.9)	
Civil servant	18(22.5)	15(18.7)	33(20.6)	
Total	**80(100)**	**80(100)**	**160(100)**	

* Chi-Square test derived value ** Fishers exact test derived value

† Significant

From table 2 below the weight, height and body mass index (BMI) are comparable in both groups. The mean weight was 64.99±11.88kg for the cases, 64.97±12.05 for the control with an overall mean weight of 64.98±11.96kg for both groups. The mean height was 1.61±0.06m for the cases, 1.60±0.06m for the control. The mean BMI for both cases and controls is in the overweight category, however, this was not statistically significant, p = 0.930.

Table 2: Anthropometric characteristics of study participants.

characteristics	**Cases** **N = 80**	**Controls** **N = 80**	**Total** **Average**	**P- value**
Weight (Kg)	64.99 ± 11.88	64.97 ± 12.05	64.98±11.96	0.992
Height (m)	1.61 ± 0.06	1.60 ± 0.06	1.61±0.06	0.875
BMI (Kg/m^2)	25.25 ± 4.17	25.20 ± 4.13	25.23±4.15	0.930

The clinical characteristics of the study participants are as shown in table 3 below. Family history of diabetes mellitus (DM) and hypertension (HTN) and BMI ≥30kg/m^2 (obesity) were not significant in both cases and controls

Table 3: Clinical Characteristics of study participants

Characteristics	Cases N = 80(%)	Controls N = 80(%)	Total N =160(%)	p-value
Family history of DM				
YES	6(7.5)	3(3.7)	9(5.6)	
NO	74(92.5)	77(96.3)	151(94.4)	0.495**
Family history of HTN				
YES	8(10.0)	2(2.5)	10(6.3)	0.098**
NO	72(90.0)	78(97.5)	150(93.6)	
Obesity				
YES	11(13.7)	12(15.0)	23(14.4)	0.870*
NO	69(86.3)	68(85.0)	137(85.6)	

* Chi-Square test derived value

** Fishers exact test derived value

From table 4 below the prevalence of insulin resistance (IR) among cases and controls using **HOMA-IR >4.5** and **FG/FI ratio <4.5** are 48.8% and 63.8% and 27.5 % and 53.8% respectively.

Table 4: Prevalence of Insulin Resistance among Cases and Controls

Characteristic	**Cases N = 80 (%)**	**Controls N = 80(%)**
Insulin Resistance(HOMA-IR >4.5)		
YES	39(48.8)	22(27.5)
NO	41(51.2)	58(72.5)
Insulin Resistance(FG:FI <4.5)		
YES	51(63.8)	43(53.8)
NO	29(36.2)	37(46.2)

HOMA-IR - Homeostasis Model Assessment of Insulin Resistance
FG/FI ratio - Fasting blood Glucose - Fasting Insulin ratio

Table 5 below shows the mean concentrations of fasting blood glucose (FG) and fasting insulin (FI) and mean values of calculated HOMA-IR and FG/FI ratio for both cases and controls. There was a significant difference between the mean fasting blood glucose of cases and controls, $p < 0.001$. No significant difference was observed in the mean HOMA-IR, FG/FI ratio and fasting insulin concentrations of both groups, even though they were higher among the cases.

Table 5: Comparison of mean values of Biochemical data using independent student t-test.

Biochemical data	Cases N = 80(%)	Controls N =80(%)	Total average N = 160(%)	p-value
FG(mmol/L)				
Mean±SD	4.77±1.14	3.58±0.78	4.18±0.96	<0.001†
FG(mg/dl)				
Mean±SD	85.79±20.51	64.39±13.95	75.09±17.23	<0.001†
FI(mIU/L)				
Mean±SD	26.62±14.62	25.15±13.61	25.89±14.12	0.509
FG/FI ratio				
Mean±SD	4.47±3.15	3.96±2.21	4.23±2.68	0.233
HOMA-IR				
Mean±SD	3.76±2.13	3.20±1.79	3.48±1.96	0.074

The converting factor from mmol/L to pmol/L is 6.945.

† Significant

Table 6 below, is a 2 x 2 contingency table of association between insulin resistance and recurrent first trimester miscarriages. There was a statistically significant difference in insulin resistance between cases and controls using HOMA-IR, p = 0.009. Using FG/FI ratio insulin resistance was detected in higher proportion among cases compared to the controls, 63.8% vs 46.2% respectively, this difference was however, not statistically significant p = 0.261.

Table 6: Test of association between insulin resistance and recurrent miscarriages in cases and controls using Chi-Square test.

characteristic	Cases N = 80(%)	Controls N = 80(%)	Total N = 160(%)	p-value
HOMA-IR (>4.5)				
YES	39(48.8)	22(27.5)	61(38.1)	
NO	41(51.2)	58(72.5)	99(61.9)	0.009†
FG/FI (<4.5)				
YES	51(63.8)	43(46.2)	94(58.8)	
NO	29(36.2)	37(53.8)	66(41.2)	0.261

† Significant

CHAPTER SIX

6.1 DISCUSSION

Recurrent spontaneous miscarriages remain a major challenge to gynaecologist. Insulin resistance is one of the treatable factors that have been implicated in the aetiologies of recurrent spontaneous miscarriages in recent studies. However, there are insufficient and conflicting evidence in the literature regarding the role of insulin resistance in the occurrence of miscarriages.

This study was undertaken to determine the prevalence of insulin resistance among women with first trimester recurrent spontaneous miscarriages and women with ongoing first trimester normal pregnancies and to determine the relationship between first trimester recurrent spontaneous miscarriages and insulin resistance.

In this study homeostasis model assessment of insulin resistance (HOMA-IR) and fasting blood glucose-fasting insulin ratio (FG/FI) of >4.5 and <4.5 respectively identified women with insulin resistance in both cases and controls.[5,6,16,19] Although there are no universally accepted reference values for classifying individuals as insulin resistant[47], the 25 percentile of a given population with the lowest insulin sensitivity or the highest Insulin resistance is generally recommended .[48, 49]

From the results obtained, the prevalence of insulin resistance among women with first trimester recurrent spontaneous miscarriages and women with normal first trimester pregnancies using HOMA-IR score was 48.8% and 27.5% respectively. This observed difference with higher prevalence among cases was statistically significant, p-value = 0.009. These results are comparable with the findings of Wani

AA et al.[50] and Craig LB et al.[5] where prevalence of 24.0% and 21.6% in the case groups and 8.0% and 8.1% among control groups were reported respectively. These differences were statistically significant with p values of 0.003 and 0.04 respectively. The results of this study, also are consistent with the study of Tian et al. among women who had assisted reproductive technology treatment for infertility. Insulin resistance status was determined before treatment as HOMA-IR >4.5. Among women that had miscarriages following treatment 47.8% were insulin resistant against 9.5% of women who had no insulin resistance but later had miscarriage.[6] In a large systematic review and meta-analysis conducted by Li ZL, et al.[12] 7 studies between 1996 and 2012 were included, with a total of 467 women with recurrent miscarriages and 413 control women. The authors found a significantly higher proportion of women with HOMA-IR >4.5 and glucose-to-insulin ratio < 4.5 among women with recurrent miscarriages. Diejomaoh et al.[19] got a prevalence of 22.9% and 6.7% among Kuwait women with history of recurrent spontaneous miscarriages of unknown aetiology and normal pregnancies respectively. This however, was not statistically significant p = 0.093.

Insulin resistance measured in terms of FG/FI ratio detected insulin resistance in 63.8% and 53.8% of cases and controls respectively. Although, IR detection was higher in the case group as compared to controls, the difference was not statistically significant, p = 0.261. These findings are consistent with the insulin resistance frequency reported by Maryam et al.[16]among Iranian women with recurrent early pregnancy loss in which the difference between absolute and proportional frequency of patients with FG to FI ratio of < 4.5 and ≥ 4.5 in case and control groups was not

significant, p=0.123. Craig LB et al.[5] also reported similar findings of 21.6% vs 9.5% for cases and controls respectively, p = 0.07

The higher prevalence of IR recorded in this study may be related to the use of same cut-off for insulin resistance across different populations. As observed earlier, there are no universally accepted reference values for all races for classifying individuals as insulin resistant[47], the 25th percentile of a given population with the lowest insulin sensitivity or the highest Insulin resistance is generally recommended .[48, 49] Also Another plausible explanation for the observed difference may be related to genetic and metabolic profile that varies across populations.

In our study there was no statistical significant difference between the two groups with respect to age and BMI. The mean age in the study group was 28.09±6.14 years and in control group was 28.10+6.21 years with p value of 0.990 which is statistically insignificant. These observations were comparable with the study of Wani AA et al.[50] and Craig LB et al.[5] where the mean ages in the case group were 28.40±2.37 and 32.70±5.40 years and in control group were 29.10±2.70 years and 32.80±6.00 years respectively. The mean BMI in the case group was 25.25± 4.17kg/m^2 and in the control group was 25.20±4.13kg/m^2 with an insignificant p value of 0.930. The results obtained were similar to the study of Craig LB et al.[5] where mean BMI was 29.20±7.30kg/m^2 in the study group and 29.00±7.20kg/m^2 in the control group. These results were also consistent with the study of Wani AA et al.[50] where the mean BMI in the study group was 23.90±2.15kg/m^2 and in the control group was 23.5±2.07kg/m^2. In the current study statistically significant difference was noted between two groups with respect to mean parity that is 0.61±0.68 in the case group and 1.58±0.92 in the control group with p value of less

than 0.001 which is statistically significant. Similar results were obtained in the study of Diejomaoh M et al.[19] where the mean parity in the case group was 0.70±0.70 and control group was 1.70±0.50. Another study by Craig LB et al.[5] showed mean parity of 0.50±0.70 in the study group and 1.70±0.70 in the control group which were also comparable with the current study. This was because majority of patients in the case group had not carry any pregnancy to the age of viability.

Significant difference was noted between the mean fasting blood glucose of cases and controls with a higher concentration in the cases group, 4.77±1.14mmol/L vs 3.58±0.78mmol/L respectively, p <0.001. The results were consistent with the study of Wani AA et al.[50] and Tian et al.[6] where mean fasting blood glucose in the study group was 5.36±0.44mmol/L and 5.31±0.43mmol/L and in control group it was 4.84±0.64mmol/L and 4.98±0.57mmol/L respectively.

The difference in mean gestational ages was not significant between the two groups, 10.68±1.52 weeks vs 11.19±1.87 weeks for cases and controls respectively, p = 0.066. This is consistent with the results of Tamara et al.[51] in which mean gestational ages of 7.20±0.80 and 7.50±0.60 weeks were reported among cases and controls respectively with a p value of 0.615.

Insulin resistance was found to be significantly associated with first trimester recurrent spontaneous miscarriages in this study, p = 0.009. The mechanisms by which insulin resistance causes miscarriage is not clearly known. One hypothesis is that insulin resistance causes uncontrolled diabetic-like state in the foetal environment resulting in increased first trimester loss.[5] High insulin levels have been shown in vitro to increase the transport of glucose by first trimester cytotrophoblast

independent of the glucose level (probably by up regulation of the GLUT-1 transport system).[52]A second possibility involves plasminogen activator inhibitor (PAI-1).Insulin resistance has been demonstrated to increased expression of PAI-1.[18]It has been suggested that increased plasminogen activator inhibitor promote recurrent abortion through thrombotic induction of placenta insufficiency. Other postulated pathways are hypercoagulable state (impaired fibrinolysis), increased inflammatory cytokine levels and decreased expression of glycodelin[24] and insulin-like growth factor binding protein-1 (IGFBP-1) and increase levels of androgens. Insulin resistance is known to play a critical role in the ovarian androgen excess and therefore might promote miscarriage by increasing circulating testosterone concentrations. The expression of glycodelin and of IGFBP-1 is decreased by the hyperinsulinemia at the implantation site.[23]Glycodelin plays an immune role, inhibiting the endometrial response towards the embryo, while the IGFBP1 facilitates the adhesion process of the blastocyst at the foetomaternal interface.

6.2 CONCLUSION

This study suggests that women with recurrent first trimester miscarriages are more likely to be insulin resistant with higher fasting blood glucose levels compared to normal controls.

6.3 LIMITATIONS OF THE STUDY

1. This was hospitals-based study; therefore the findings may not reflect the findings in the entire population of women with first trimester recurrent spontaneous miscarriages in Nigeria.
2. Inability to exclude genetic causes of first trimester miscarriages.

6.4 RECOMMENDATIONS

1. Women with recurrent first trimester miscarriages presenting for evaluation should have their oral glucose tolerance test (OGTT) assessed.
2. Women with recurrent miscarriages of unknown cause should be assessed for insulin resistance as it may require treatment which includes life style changes, exercise and insulin sensitizing drugs like metformin.
3. A larger local study should be done to determine HOMA-Index cut-off that defines insulin resistance in our population.

REFERENCES

1. Jeve YB, Davies W. Evidence - based management of recurrent miscarriages. J Hum Reprod Sci. 2014; 7 (3): 159-169.
2. News S, Smith ML, Schust DJ. Endocrinology and Recurrent Early Pregnancy Loss. Semin Reprod Med. 2011; 29 (6): 482-490.
3. Ford HB, Schust DJ. Recurrent Pregnancy Loss : Aetiology, Diagnosis and Therapy. Rev Med Reviews Gynecol. 2009; 2 (2): 76–83.
4. Practice T, Medicine R. Evaluation and treatment of recurrent pregnancy loss : a committee opinion. Fertil. Steril. 2012; 98: 1103–1111.
5. Craig LB, Ke RW, Kutteh WH. Increased prevalence of insulin resistance in women with a history of recurrent pregnancy loss. Fertil. Steril. 2002; 78 (3): 487-490.
6. Tian L, Shen H, Lu Q, Norman R J, Wang J. Insulin Resistance Increases the Risk of Spontaneous Miscarriages. J Clin Endocrinol Metab. 2007; 92 (4): 1430– 1433.
7. Ispasoiu C, Chicea R, Stamatian FV, Ispasoiu F. High Fasting Insulin Levels and Insulin Resistance May Be Linked to Idiopathic Recurrent Pregnancy Loss : A Case-Control Study. Inter. J Endocr. 2013: 1-4.
8. Li TC, Marleen DE, Elizabeth T, Barbara A, Martin L, Susan L. Endocrinological and Endometrial Factors in Recurrent Miscarriages. Br J Obstet Gyne. 2000; 107: 1471-1479.
9. Banu J, Fatima P, Sultana P, Chowdhury MA, Begum N, Anwary SA, Ishrat S, Deeba F, Begum SA. Association of infertile patients having polycystic ovarian

syndrome with recurrent miscarriage. Mymensingh Med J. 2014; 23 (4): 770-3.

10. Direction S, Fanti VM. Health and fertility in World Health Organization group 2 anovulatory women. Human Reproduction Update. 2012; 18 (5): 586-599.
11. Wang Y, Huidan Z, Yan L, Jianping Z, Jiangping T, Yukun L. Relationship between Recurrent Miscarriages and Insulin Resistance. Gynecol Obstet. 2011; 72: 245–251.
12. Li ZL, Xiang HF, Cheng LH, Cao YX, Wei ZL, Liu C et al. Association between recurrent miscarriages and insulin resistance, a meta-analysis. Zhonghua Fu Chan KeZaZhi. 2012; 47 (12): 915-9.
13. Hong Y, Xie QX, Chen CY, Yan C, Li YZ, Chen DM, Xie MQ. Insulin resistance in first-trimester pregnant women with pre-pregnant glucose tolerance and history of recurrent spontaneous abortion. J Biol Requl Hemeost Agent. 2013; 27 (1): 225-31.
14. Glueck C J, Wang P, Goldenberg N, Sieve-Smith L. Pregnancy outcomes among women with polycystic ovary syndrome treated with metformin. Hum. Reprod. 2002; 17 (11): 2858–2864.
15. Palomba S, Angela F, Francesco O, Tiziana R, Achille T, Fulvio Z. Metformin hydrochloride and recurrent miscarriage in a woman with polycystic ovary syndrome. Fertil. Steril. 2006; 85 (5): 1511-5.
16. Maryam K, Bouzari Z, Basirat Z, Kashifard M, Zadeh MZ. The comparison of insulin resistance frequency in patients with recurrent early pregnancy loss to normal individuals. BMC Res. Notes. 2012; 5: 133-138.

17. Chakraborty P, Goswami SK, Rajani S, Sharma S, Kabir SN. Recurrent Pregnancy Loss in Polycystic Ovary Syndrome : Role of Hyperhomocysteinemia and Insulin Resistance. PLoS ONE. 2013; 8 (5): 1–6.

18. Declaration H, Experimenta H, Graecia M. Plasminogen activator inhibitor 1 and miscarriage after metformin treatment and laparoscopic ovarian drilling in patients with polycystic ovary syndrome. Fertil. Steril. 2005; 84 (3): 761-765.

19. Diejomaoh M, Al-azemi M, Jiri J, Madhu G, Manal A, Rasheeda F. Insulin Resistance in Women with Recurrent Spontaneous Miscarriages of Unknown Aetiology. Med Princ Pract. 2007; 16: 114-118.

20. Cocksedge K A, Saravelo SH, Wang Q, Tuckerman E, Laird SM, Li TC. Does free androgen index predict subsequent pregnancy outcome in women with recurrent miscarriage ? Hum Reprod. 2008; 23 (4): 797–802.

21. Nawrocka-rutkowska J, Ciećwież S, Marciniak A, Agnieszka B, Berenika W, Daruisz K. Insulin resistance assessment in patients with polycystic ovary syndrome using different diagnostic criteria – Impact of metformin treatment. Ann Agric Environ Med. 2013; 20 (3): 528–532.

22. Palomba S, Falbo A, Orio F, Fulvio Z. Polycystic Ovary Syndrome Effect of Preconceptional Metformin on Abortion Risk in Polycystic Ovary syndrome : a systematic review and meta-analysis of randomized controlled trials. Fertil. Steril. 2009; 92 (5): 1646–1658.

23. Nagaev I, Bokarewa M, Tarkowski A, Smith U. Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. PLoS One 1. 2006; 1(1):1-9.

24. Jakubowicz D J, Paulina AE, Makku S, Salomon J, Jean-Patrice B, Riitta K et al. Reduced Serum Glycodelin and Insulin-Like Growth Factor-Binding Protein-1 in Women with Polycystic. J Clin. Endocrinol. Metab. 2004; 89 (2): 833–839.

25. Seppala M, Tarloy RN, Koistinen H, Kointinen R, Milgrom E. Glycodelin: a Major lipocalin protein of the reproductive axis with diverse action in cell recognition. Endocr. Rev. 2002; 23 (4): 401–43.

26. Meigs JB, Paul FJ, Jacob S, Daniel ES, David MN, Nader R et al. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham offspring study. Diabetes Care. 2001; 24 (8): 1403–1410.

27. Schachter M, Ariel R, Devorah S, Carmela R, Raphael R, Shevach F. Prospective, randomized trial of metformin and vitamins for the reduction of plasma homocysteine in insulin-resistant polycystic ovary syndrome. Fertil. Steril. 2007; 88 (1): 227–230.

28. Jakubowicz DJ, Iuorno MJ, Jakubowicz S, Roberts KA, Nestler JE. Effects of metformin on early pregnancy loss in the polycystic ovary syndrome. J. Clin. Endocrinol. Metab. 2002; 87 (2): 524–529.

29. Eisenhardt S, Henschel V, Germeyer A, Wolff M, Hamann A, Strowitzki T. Early effects of metformin in women with polycystic ovary syndrome: a prospective randomized, double-blind, placebo-controlled trial. J. Clin. Endocrinol. Metab. 2006; 91 (3): 946–952.

30. Dhindsa G, Bhatia R, Dhindsa M, Bhatia V. Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome. J Postgrad Med. 2004; 50 (2): 140-144.

31. Zolghadri J, Tavana Z, Kazerooni T, Soveid M, Marzie T. Relationship between abnormal glucose tolerance test and history of previous recurrent miscarriages, and beneficial effect of metformin in these patients : a prospective clinical study. Fertil. Steril. 2008; 90 (3): 727–730.

32. Mcbride KL, James PB. Recurrent miscarriages. InnovAiT. 2014; 7 (1): 25–34.

33. Okonofua FE. Abortion. In: Okonofua F and Odunsi K (Eds), Contemporary Obstetrics and Gynaecology for Developing Countries. Women's Health and Action. Research Centre. 2003; 179-201.

34. Catriona MS. Spontaneous Miscarriage. In: Edmonds DK (Ed), Dewhurst's Textbook of Obstetrics and Gynaecology 8th Edition. UK: Blackwell Publishing. 2012; 6: 53-59.

35. John CP, Inna B, Francisco T, Carl VS. Recurrent Early Pregnancy Losses Practice Essentials, Overview, Incidence ALL. Medscape. 2014.

36. Practical Committee of the American Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy losses: A committee opinion. Fertil. Steril. 2013; 99 (1): 63.

37. Queensland Clinical Guideline. Early pregnancy loss. @ www.health.qld.gov.au/qcg. 2015; 1-33.

38. Peter Kovacs. The Best Strategies for Managing Women with Recurrent Pregnancy Losses. Medscape. 2013, Dec 18.

39. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil. Steril. 2005; 83 (5): 1454–1460.

40. News S, Kovacs P. Highlights of the American Society for Reproductive Medicine (ASRM) 62nd Annual Conference. Medscape. 2007, April 18.

41. Tasoula T, Caroline O, Gerard C. The Pathophysiology of Polycystic Ovary Syndrome. Clin. Endocrinol. 2004; 60 (1): 1–3.

42. Carrington B, Sacks G, Regan L. Recurrent miscarriages: pathophysiology and outcome. Curr. Opin. Obstet. Gynecol. 2005; 17(6): 591–7.

43. Kirsten AM, Jim IM, Geoffrey JC, Thomas FI, Geoffrey MS. Point: HOMA Satisfactory for the Time being. Diabetes Care. 2007; 30 (9): 2411-2413.

44. Borai A, Livingstone C, Ferns GA. The Biochemical Assessment of Insulin Resistance. Ann Clin Biochem. 2007; 44 (4): 324-342.

45. Wallace TM, Levy JC, Matthews DR. Use and Abuse of HOMA modelling. Diabetes Care. 2014; 27: 1487-1495.

46. Esteghamati A, Ashraf H, Khalilzadeh O, Zandieh A, Nakhjavani M, Rashidi A. Optima Cut-off of homeostasis model assessment in insulin resistance (HOMAIR) for the diagnosis of metabolic syndrome: Third National Surveillance of the Risk Factors of Non-communicable diseases in Iran (SuRFNCD-2007). Nutrition & metabolism. 2010, 7: 26.

47. Levy JC, Matthews DR, Herman MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program (Letter). Diabetes Care. 1998; 21: 2191-2192.

48. Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Shulman GI et al. Proglitazone Improves Insulin Sensitivity among Non-diabetic Patients with a Recent Transient Ischemic Attack or Ischemic Stroke. Stroke. 2003; 34:1431-1436.

49. Reaven GM, Hollenbeck CB, Chen YD. Relationship between glucose intolerance, insulin secretion, and insulin action in non-obese individuals with varying degrees of glucose intolerance. Diabetologia l989; 32:52-55.

50. Wani AA, Gul I, Jabeen F, Kaul S, Lone FA, Akhter G. Relationship of insulin resistance with recurrent pregnancy loss. Int J Reprod Contracept Obstet Gynecol 2017; 6:1312-7.

51. Tamara T, Elkholy A, Elsaed N, Abdellatif M, Essam N, Mohamd S. Association between Unexplained Recurrent Miscarriage and Insulin Resistance. Int J Reprod Med Gynecol. 2018; 4(1): 001-005.

52. Gordon MC, Zimmerman PD, Landon MB, Gabbe SG, Kniss DA. Insulin and glucose modulate glucose messenger ribonucleic acid expression and glucose uptake in trophoblasts isolated from first-trimester chorionic villi. Am J Obstet Gynecol. 1995; 173:189–97.

APPENDIX A

INFORMED CONSENT

I am Dr Adikpe Emmanuel Edugbe, a Senior Registrar with the department of Obstetrics and Gynaecology Jos University Teaching Hospital.

I am carrying out a study on the relationship of insulin resistance among women with unexplained pregnancy loss compared with normal pregnant women at Jos University Teaching Hospital. Insulin resistance and compensatory hyperinsulinemia have been reported to pose significant threat to the success of pregnancy. They have been implicated in women with two or more unexplained pregnancy loss at ≤13 weeks of gestation.

The association between unexplained pregnancy loss and insulin resistance is lacking in Jos and Northern Nigeria. This study is aimed at determining this association in other to manage these patients better.

The study is in partial fulfilment of the requirement for Part II examinations of the National Postgraduate Medical College of Nigeria.

You will be required to fast for 8 to 12 hours and five millilitres (5ml) of your blood will be taken for serum estimation of fasting glucose and fasting insulin. I will pay for the laboratory test. You will feel a little pain while the sample is being collected, but it is temporary. Any information that will be provided for this study will be treated as confidential.

The study is voluntary and you should feel free to participate or decline. Your decision not to participate in this study will not affect your care in the hospital. All information provided will be strictly confidential and your identity would not be disclosed.

I (initials please)……..understand the purpose of the study and I volunteer to participate in the study. Thank you.

Signature of patient…… Date……..

Signature of witness…….. Date……..

Signature of investigator…….. Date……..

APPENDIX B

QUESTIONNAIRE

ASSOCIATION BETWEEN INSULIN RESISTANCE AMONG WOMEN WITH EARLY SPONTANEOUS MISCARRIAGE AND WOMEN WITH NORMAL PREGNANCY IN JUTH

	DD	MM	YY										
DATE					**SERIAL NUMBER**		**HOSPITAL NUMBER**						

CASES (A) CONTROLS (B)

1. Age (Years)…….
2. Ethnicity (a) Hausa/Fulani (b) Igbo (c) Yoruba (d) others (specify) ……
3. Level of education (a) none (b) primary (c) secondary (d) tertiary
4. Occupation (a) housewife (b) student (c) trader (d) self-employed (e) Civil servant (f) Others (specify) …….
5. Parity…….
6. Gestational age at current pregnancy loss (weeks) (a) <10 weeks (b) 10 – 13 weeks
9. Gestational age at previous pregnancy loss 1)..... 2)..... 3)..... 4).....
10. Height (m)……
11. Weight (Kg)…….
12. BMI (Kg/m^2)……..
13. Family history of diabetes mellitus (a) Yes (b) No
15. History of macrosomic babies (a) Yes (b) No