Environmental Influences in Normal Weight Women with History of Recurrent Pregnancy Loss

O.B. Aborisade [1], M.A. Charles-Davies[2*], M.A. Okunlola[3]

[1,2]Department of Chemical Pathology
[2]Department of Obstetrics and Gynaecology
College of Medicine,University College Hospital, Ibadan.

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Aim: The study investigated the possible relationship of luteal phase progesterone, toxic heavy metals and nutrients in normal-weight women with history of recurrent pregnancy loss for adequate management.
Study Design: Cross sectional study
Place and Duration of Study: The Obstetrics and Gynaecology Clinic, University College Hospital, Ibadan, Olabisi Onabanjo University Teaching Hospital, Shagamu and State Hospital, Ijebu-Ode; the University of Ibadan and environs between April and September, 2009.
Methodology: 90 apparently healthy women with normal weight aged 18-45years with regular and ovulatory cycles of 26-30 days were enrolled. They were 60 women with history of recurrent pregnancy loss (cases) age-matched with 30 women without history of recurrent pregnancy loss (controls). Demographic and anthropometric measurements were done by standard methods. Progesterone was determined by enzyme immunoassay (immunometrics UK Ltd). Total cholesterol, triglyceride and high density lipoprotein were performed by enzymatic methods (Randox laboratories, USA) while low density lipoprotein was calculated using Friedwald's formula. Zinc, selenium, chromium, manganese, iron, magnesium, copper, lead, and cadmium were estimated by atomic absorption spectrophotometry while Vitamin E was measured by high performance liquid chromatography. Statistical analysis was done using SPSS version 16.0.
Results: Results showed significantly higher levels of triglycerides, cadmium and lead, and significantly lower levels of progesterone, iron, copper, magnesium, chromium, selenium and vitamin E in cases compared with controls ($p<0.013$).
Conclusion: Oxidative stress mechanisms in normal-weight women with history of recurrent pregnancy loss were implicated. Healthcare policies should focus on pollution reduction and increase awareness on healthy diet for optimal periconceptional micronutrient requirements.

Keywords: Progesterone, metabolic syndrome, micronutrients, endocrine disruptors, recurrent pregnancy loss

1. INTRODUCTION

Early pregnancy loss is one of the most common disorders of human pregnancy and a public health concern with risk factors that have not been elucidated (Jauniaux et al.,2006; Ruder et al., 2008; Caserta et al., 2008). Recurrent pregnancy loss (RPL), defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation affects 0.5%–3% of women in the reproductive age group. 50%–60% of these pregnancies are idiopathic and highly frustrating for both patients and physicians (Gupta et al., 2007).

Advanced maternal age (>35yrs), obesity, increased number of previous pregnancy, and luteal phase insufficiency with reduced progesterone levels are known risk factors in the aetiology of RPL with less clearly defined mechanisms (Lashen et al., 2004; Romero, 2007; Metwally et al., 2008, 2010; Kroon et al., 2011). Recent reports implicate environmental and lifestyle factors in RPL (Ruder et. al., 2008; Caserta et. al.,2011).

Cadmium (Cd) and lead (Pb) are toxic heavy metals (Jomova et al., 2011; Caserta et al., 2011), associated with several modern industrial processes and obtained through the food chain (Thompson, Bannigan, 2008; Caserta et al., 2011). Accumulation of heavy metals like Cd and Pb in African cat fish (Clarias gariepinus) from Ogun River near six major industries in the region of this present study has been observed (Farombi et al., 2007). Cd and Pb disrupt endocrine function and are recognized risk factors for hormonal changes, impaired placental development, congenital malformations, fetal growth restriction and spontaneous abortion (Caserta et. al., 2011). The negative impact of heavy metals on pituitary and ovarian functions has been reported (Gerhard et al.,1998). Correlations of heavy metal excretion with different hormones- progesterone, oestradiol, prolactin, thyroid stimulating hormone have been reported (Gerhard et al.,1998).

Disruption of metal ion homeostasis leads to increased formation of potentially destructive reactive oxygen species (ROS), which overwhelms body antioxidant protection. The resultant oxidative stress (OS) subsequently induces DNA damage, lipid peroxidation, protein modification and eventual cellular damage (Tremellen, 2008; Jomova et al., 2011). Increased free radical activity has been shown in the pathogenesis of recurrent abortion (Vural et al., 2000). Systemic and placental oxidative stress have been implicated in the pathophysiology of RPL. Depletion of antioxidant defences caused by increased oxidative stress has been demonstrated in RPL (Vural et al.,2000; Gupta et al.,2007; Ruder et al., 2008). Diet, particularly its constituent antioxidants, and oxidative stress may influence the timing and maintenance of a viable pregnancy (Ruder et al., 2008). Thus micronutrient deficiencies might affect fertility, embryogenesis and placentation and their prophylactic use in preventing these adverse pregnancy outcomes have been advocated (Cetin et al., 2010). This study was designed to understand the roles of heavy metals and nutrients and their specific influences on progesterone in normal weight women with RPL to elucidate underlying mechanisms for both prevention and informed management
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2. MATERIAL AND METHODS

2.1 Subjects

A total of 90 apparently healthy women with normal body mass index (BMI) of 18.5-24.9 kg/m^2 (WHO, 1998) with regular and ovulatory cycles of 26-30 days aged 18-45 years were recruited after informed consent for this prospective cross sectional study using stratified random sampling method. These were 60 non-pregnant women with history of recurrent abortion and at least 3 pregnancy losses before 20 weeks of gestation (cases) age matched with 30 non-pregnant women without history of recurrent abortion with at least one child

(controls). All cases had normal values of luteinising hormone, and normal transvaginal/ abdominal scan done recentlly without fibroid, polycystic ovarian syndrome or abdominal mass. None of the women was on lipid lowering or hormonal drugs. Individuals with cardiovascular diseases, diabetes and renal failure and those who did not give consent were excluded from the study. The cases were selected at the Obstetrics and Gynaecology Department of the University College Hospital, Ibadan (UCH), Olabisi Onabanjo University Teaching Hospital, Shagamu and State Hospital, Ijebu-Ode while controls were selected from University of Ibadan and environs. Ethical approval was obtained from the University of Ibadan/UCH Ethical Board.

2.2 Collection of blood

10ml of fasting venous blood sample was aseptically taken from the antecubital fossa vein with minimal stasis on day 21 of the menstrual cycle of all women. 5ml was dispensed into ethylene diamine tetra-acetic acid (EDTA) tubes for the estimation of lipids while the remaining 5ml were dispensed into plain tubes and kept for 1-2 hours to clot for the estimation of micronutrients, heavy metals and progesterone. The samples were then centrifuged at 500g for 5mins to obtain plasma and serum respectively, and stored at -20ºC until analysis.

2.3 Demographic Characteristics

Semi structured pre-test questionnaire was completed by each subject in order to obtain demographic data. The demographic characteristics tested were age and age at menarche (AM), length of menstrual cycle (LMC), number of previous pregnancies (NPP) and number of live birth(s) (NLB).

2.4 Anthropometric Characteristics

The anthropometric parameters measured were weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), and waist hip ratio (WHR) according to methods described by Umoh et. al., 2010.

2.5 Biochemical Characteristics

The biochemical variables tested were progesterone, lipids[triglyceride (TG); high density lipoprotein (HDL), total cholesterol (TC) and low density lipoprotein-cholesterol (LDL)], micronutrients[zinc (Zn), iron (Fe), copper(Cu), manganese (Mn), magnesium(Mg), chromium (Cr) and selenium (Se) and Vitamin E] and heavy metals[lead (Pb) and cadmium (Cd)]. Progesterone was estimated by enzyme immunoassay method (Pugeat et. al., 1981) (Immunometrics UK. Ltd.). Total cholesterol was estimated by enzymatic method (Alain et al.1974). TG was analysed by method described by Trinder, 1969. HDL was estimated by precipitating low density lipoprotein cholesterol; very low density lipoprotein cholesterol and chylomicron fractions using phosphotungistic acid in the presence of magnesium ions. The HDL cholesterol which remained in the supernatant was estimated using the total cholesterol method. Low density lipoprotein cholesterol was calculated using formula of Friedwald et. al.(1972). All analyses of lipid fractions were done by commercially produced kits (Randox Laboratories, USA). Micronutrients(Zn, Fe, Cu, Mn, Mg, Cr, Se) and heavy metals(Pb and Cd) were estimated by Atomic Absorption Spectrophotometer(Beck 200) based on the direct method described by Kaneko (1999) while vitamin E was estimated by high performance liquid chromatography (Waters, USA).

2.6 Statistical Analysis

Statistical package for social scientist (SPSS) version 16.0 was employed for the analyses of data. Student's t –test was used for the comparison of quantitative variables. Pearson's correlation coefficient was used for the relationship between quantitative variables. $p<0.05$ was regarded as significant.

3. RESULTS AND DISCUSSION

3.1 Lipids, Progesterone, Demographic and Anthropometric Characteristics

Table 1 shows the comparison of lipids, progesterone, demographic and anthropometric characteristics between women with recurrent abortion (cases) and controls. Of all parameters tested, only NPP and TG were significantly higher while progesterone and NLB were significantly lower in cases compared with controls ($p<0.0001$). The steroid hormone, progesterone is critical in maintenance of pregnancy (Gellerson et al., 2009) and luteal phase insufficiency with low progesterone production is an established endocrine cause of RPL (Dhont, 2003; Romero, 2007). Other risk factors are advanced maternal age (>35yrs) and obesity (Kroon et al., 2011). In this present study of non-obese women (aged <35yrs with cases age-matched with controls), our findings confirm earlier reports (Dhont, 2003; Romero, 2007; Kroon et al., 2011) and suggests that TG, NPP, NLB and progesterone may be independent of obesity and age as risk factors of RPL.

Cholesterol is the precursor of all steroid hormones and impairment of lipid metabolism has long been implicated in the luteal phase deficiency of progesterone resulting in RPL (Coste et al.,1991). It is hypothesised that cholesterol insufficiency impedes the initiation of sex hormones resulting in foetal loss (Shekeres-Bartho et. al., 1990). Our data showed no significant differences in cholesterol containing lipid fractions-TC, LDL and HDL ($p>0.318$) between cases and controls. However, the observed higher TG levels in the cases compared with controls in this study, contrary to earlier findings of Oladimeji et al. (2002) in our geographical sub-region, implicates current lifestyle factors. Metabolic syndrome characterised by increased levels of triglycerides, abdominal obesity and insulin resistance is now prevalent in Nigeria (Charles-Davies et al., 2012). This has been attributed to changes by Nigerians to western diet and lifestyle (Utinwa, 2009; Adegoke et al., 2010) and might contribute to RPL. Obesity and/or insulin resistance have been associated with an increased risk of pregnancy loss (Dhont, 2003).

3.2 Micronutrients and Heavy metals

Table 2 shows the comparison of micronutrients and heavy metals between women with recurrent abortion (cases) and controls. Cd and Pb were significantly higher in cases compared with controls ($p<0.013$). Cd and Pb are known to accumulate in fish (Farombi et. al., 2007). Consumption of fish as well as continuous and prolonged exposure to Cd and Pb have been linked to adverse pregnancy outcomes (Carserta et. al.,2011). The overall effect of toxic metals on any cell or tissue has been attributed to a synergy of several mechanisms with one mechanism predominating in a specific cell type (Thompson, Bannigan, 2008). Chemical pollutants have been shown to interact additively to diet and lifestyle to modulate endocrine function. As endocrine disruptors, Cd and Pb target the homeostasis of sex steroids through multiple diverse pathways. (Thompson, Bannigan, 2008; Caserta et. al., 2011).

Oxidative stress has been implicated as one of the main underlying mechanisms in the pathogenesis of spontaneous abortion (Gupta et al.,2007; Ruder et. al., 2008). Increased

Table1

Comparison of Demographic, Anthropometric, Lipids and Progesterone between Women with Recurrent Abortion (cases) and controls

Parameters	Cases n = 60	Controls n = 30	p-value
Demographic Parameters			
Age (years)	34.37±0.55	33.97±4.31	0.750
AM (years)	13.92±0.19	17.23±3.34	0.165
LMC (days)	28.45±0.40	28.77±1.48	0.596
NLB	01.43±0.12	02.67±0.14	0.000**
NPP	04.67±0.13	02.67±0.13	0.000**
Anthropometric Parameters			
Height (m)	01.70±0.02	01.68±0.02	0.542
Weight (kg)	64.22±0.66	64.70±0.71	0.650
BMI (kg/m^2)	22.74±0.43	22.87±0.54	0.860
WC (cm)	16.93±0.26	16.93±0.31	1.000
HC (cm)	47.80±0.47	46.73±0.50	0.163
WHR	0.353±0.003	0.359±0.004	0.219
Lipids			
TC (mg/dl)	139.15±5.00	153.50±3.80	0.061
TG (mg/dl)	93.15±3.41	90.67±5.38	0.025*
HDL (mg/dl)	45.30±0.64	46.40±0.89	0.318
LDL (mg/dl)	75.55±4.94	88.97±4.28	0.082
Progesterone (nmol/L)	04.84±0.61	18.35±3.09	0.000**

*= significant, n=no. of subjects, T-test=student's t-test, p = probability, cases= Non-pregnant women with history of recurrent abortion, controls= Non-pregnant women without history of recurrent abortion, BMI= body mass index, WHR= waist hip ratio, LMC=length of menstrual cycle, AM= age at menarche, NLB= number of live births, NPP= number of previous pregnancy HC= hip circumference, WC= waist circumference, TC=total cholesterol, TG=triglyceride, HDL=high density lipoprotein, LDL=low density lipoprotein

OS seems a common end-point to several endocrine disruptors (Caserta et. al.,2011). Loss of antioxidant defences, high levels of biochemical markers of ROS-induced membrane

damage such as lipid peroxidation products and oxidant/antioxidant imbalance are associated with pregnancy loss. Oxidative damage to macromolecules and DNA and ROS-induced signal transduction for various genes might result in recurrent pregnancy loss (Gupta et. al.,2007; Ruder et. al., 2008; Thompson, Bannigan, 2008).

Female OS is postulated as a likely mediator of conception and early pregnancy loss might be placenta related (Jauniaux et al., 2006; Ruder et al., 2008). The placenta is exposed to high oxygen metabolism (Gupta et al., 2007; Ruder et al., 2008). Placental OS, with associated necrosis and apoptosis of the trophoblastic epithelium of the placental villous tree has been suggested. Development of the placento–decidual interface is severely impaired leading to early and widespread onset of maternal blood flow and major oxidative degeneration, which influences the timing of early pregnancy loss (Jauniaux et al., 2006).

The mechanism of metal-induced formation of free radicals is tightly influenced by the action of cellular antioxidants (Jomova et al., 2011). The antioxidant status is known to be a physiological barrier against free radical attack in certain body compartments. Retardation of trophoblastic outgrowth and development, placental necrosis and suppression of steroid

Table 2

Comparison of Micronutrients and Heavy Metals between Women with Recurrent Abortion (cases) and controls

Micronutrients	Cases n= 60	Controls n= 30	p- value
Zinc (µmol/L)	13.45±0.80	12.24±0.43	0.308
Selenium (µmol/L)	08.46±0.21	11.95±0.36	0.000**
Manganese(µmol/L)	09.49±0.18	11.04±0.36	0.000**
Chromium (µmol/L)	11.14±0.21	12.21±0.41	0.012**
Magnesium (µmol/L)	00.75±0.04	01.04±0.03	0.000**
Iron (µmol/L)	11.42±0.67	18.36±0.77	0.000**
Vitamin E (mg/dl)	03.48±0.24	06.89±0.30	0.000**
Copper (µmol/L)	11.71±0.44	15.39±0.54	0.000**
Toxic trace metals			
Cadmium(µmol/L)	03.58±0.17	02.45±0.13	0.000**
Lead(µmol/L)	02.93±0.08	02.05±0.33	0.001**

* = significant, n = number of subjects, p = probability, Cases= Non-pregnant women with history of recurrent abortion, Controls= Non pregnant women without recurrent abortion, T- test= student's t- test

biosynthesis, and altered handling of nutrient metals by the placenta all contribute to implantation delay and possible early pregnancy loss (Thompson, Bannigan, 2008). Placental oxidative stress ultimately depletes antioxidant defences (Gupta et. al., 2007; Ruder et al., 2008). We observed significantly lower levels of micronutrients (Fe, Cu, Mn, Mg, Cr, Se, Vit.E) in cases compared with controls ($p<0.013$)Table2. Iron deficiency contributes to one of the largest prevalence of micronutrient deficiencies among pregnant women (Haider et al., 2011). Nutrients interact with endocrine disruptors to mitigate their effects. Vitamin E with antioxidant activities appear to moderate the effect of endocrine disruptors and other toxicants (Caserta et. al., 2011). The ability of Cd and Pb to imitate nutritive metals that are divalent cations-Fe, Zn, Mg and Mn in many biological pathways and processes as well as interact with membrane transporters involved in their uptake, is a possible explanation for their toxicity. Divalent nutrients have demonstrably protected embryonic and adult tissue from toxic metal induced damage. Se probably blocks Cd uptake by embryonic cells (Thompson, Bannigan, 2008). Zn, an essential component of numerous proteins involved in the defence against oxidative stress, has an impact on the immune system and possesses neuroprotective properties. Its depletion is known to impair DNA repair mechanisms and enhance DNA damage(Jomova et al,2011).The similar Zn levels in both cases in our study(Table 2), suggest synergistic action of multinutrients to prevent adverse pregnancy outcomes (Haider et. al., 2011).

3.3 Relationship of Lipids, Demograhic and Anthropometric Characteristics with Progesterone

Rapid non-genomic events might critically determine the functional response of progesterone in the female reproductive system in a cell-type- and environment-specific manner (Gellerson et al., 2009). We observed significantly positive correlations of only age and weight with progesterone in cases only ($p<0.026$). No correlation was observed between all parameters tested and progesterone in controls ($p>0.05$). Our observations suggest that increasing age and weight may predict progesterone levels in non-obese women with RPL aged below 35 years. The lack of correlation of progesterone with Cd and Pb, micronutrients and any of the lipid fractions suggest that their importance in RPL may be due to factors other than progesterone. Cadmium has recently been recognized as an estrogenic endocrine disruptor. Cd and Pb might target sex steroids other than progesterone, possibly acting on the pathways mediated by oestrogen receptors (Carserta et. al, 2011). We did not estimate levels of other hormones like oestrogen, implicated in RPL, which is the limitation of this study.

Pregnancy represents a state of increased metabolic requirements and micronutrient malnutrition among women of reproductive age and pregnant women is much more common in developing countries than in industrialized countries (Haider et al., 2011; Kawai et al., 2011). Deficient micronutrients in the non-pregnant state and/or increased micronutrient requirements during repeated pregnancies of our cases might have further exacerbated the pre-existing maternal deficiency (Haider et. al., 2011). Recommendations for prenatal micronutrient supplements are absent in most developing countries, contrary to some developed countries where such supplements are routinely recommended (Kawai et. al.,2011). Micronutrient supplementation in pregnant women particularly in developing countries is controversial (Haider et. al.,2011; Kawai et. al.,2011). Our study show the impact of the environment in addition to diet and lifestyle and progesterone in women with RPL. However, these factors may be independent causes of RPL particularly in normal weight women of less than 35 yrs with RPL. Healthcare strategies show focus on reduction of environmental pollution and increase awareness on healthy diet for optimal periconceptional micronutrient requirements (Cetin et al., 2010, Carserta et al., 2011). Tightly monitored clinical trials on the safety, efficacy and effective delivery of maternal micronutrient

supplementation in women with RPL (Kawai et al., 2012) before and during pregnancy are recommended.

4. CONCLUSION

We observed in our study that reduced progesterone levels, number of previous pregnancies, number of live births, levels of micronutrients (Iron, Copper, Manganese, Magnesium, Chromium, Selenium, Vitamin E) and increased levels of triglycerides, toxic metals (cadmium, lead) are independent risk factors for recurrent pregnancy loss in normal weight women below 35 years. Of all factors only age and weight correlated with progesterone. Our findings implicate oxidative stress mechanisms. Inadequate micronutrients in our cases might be further exacerbated in the next pregnancy due to increased micronutrient demands thus forming a vicious cycle of repeated/recurrent pregnancies losses. Healthcare strategies and research should therefore focus on reduction of environmental pollution and increase awareness on healthy diet for optimal micronutrient requirements in women with recurrent pregnancy loss.

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COMPETING INTERESTS

We declare that no competing interests exist.

AUTHORS' CONTRIBUTIONS

O.B. Aborisade participated in conception and design of the study, managed the literature searches, did the laboratory and statistical analyses of the study, interpreted data, and prepared the first draft of the study. M.A. Charles-Davies participated in the conception and design of the study, literature searches and interpretation of data, critical revision of article for important intellectual content and wrote the final version to be published.M.A.OKUNLOLA participated in the design of the study, enrolment of participants in the study and revision of article for important intellectual content. All authors read and approved the final manuscript.

CONSENT (WHEREEVER APPLICABLE)

We declare that informed consent was obtained from all the participants in the study after detailed explanation of the study.

ETHICAL APPROVAL

We hereby declare that the study protocol was examined and approved by the University of Ibadan /University College Hospital, Ibadan ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki."

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