

Environmental Influences in Non-Obese Women with History of Recurrent Pregnancy Loss

ABSTRACT

Metabolic syndrome and environmental pollution are now prevalent in Nigeria. Luteal phase deficiency of maternal progesterone, obesity, toxic heavy metals and micronutrient deficiency are implicated in recurrent abortion with unclear mechanisms. We determined the possible association of these indices in women with idiopathic recurrent abortion. The study was prospective and cross sectional in design involving 90 non-pregnant women with normal body mass index aged 18-45 years. They were 60 women with history of recurrent abortion (cases) age-matched with apparently healthy women without history of abortion (controls). Demographic and anthropometric measurements were done by standard methods. Progesterone was determined by enzyme immunoassay. Total cholesterol, triglyceride and high density lipoprotein were performed by enzymatic methods (Randox laboratories, USA) while low density lipoprotein was calculated using Friedwalds 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 show 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 ($p < 0.013$) implicating oxidative stress mechanisms. Healthcare strategies and research should focus on reduction of environmental pollution and increase awareness on healthy diet for optimal periconceptional micronutrient requirements.

Keywords: Progesterone, metabolic syndrome, micronutrients, endocrine disruptors, recurrent abortion.

INTRODUCTION

Early pregnancy loss is one of the most common disorders of human pregnancy and a significant 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 (>35 yrs), 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 as well as endocrine disrupting chemicals (Jomova et al., 2011; Caserta et al., 2011). Endocrine disruptors (EDs) are chemicals that interfere with normal signaling systems by mimicking, blocking or modulating the synthesis, release, transport, metabolism and binding or elimination of natural hormones (Caserta et al., 2011). They are 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 the Ogun River in Ogun State located close to six major industries in the South Western part of Nigeria, the location of this present study has been

reported (Farombi et al., 2007). Heavy metals seem to have a negative impact on ovarian as well as pituitary function and changes may be important factors in the pathogenesis of repeated miscarriages. Correlations of heavy metal excretion with different hormones- progesterone, oestradiol, prolactin, thyroid stimulating hormone have been reported (Gerhard et al., 1998). Cd and Pb are associated with adverse pregnancy outcomes in women with continuous and prolonged exposure (Caserta et al., 2011).

Increased free radical activity has been implicated in the pathogenesis of recurrent abortion (Vural et al., 2000). 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). Systemic and placental oxidative stress have been implicated in the pathophysiology of RPL. OS-induced damage has been demonstrated in RPL (Vural et al., 2000; Gupta et al., 2007; Ruder et al., 2008).

Depletion and weakness of antioxidant defences caused by increased oxidative stress has been demonstrated in RPL (Vural et al., 2000). Diet, particularly its constituent antioxidants, and oxidative stress may influence the timing and maintenance of a viable pregnancy (Ruder et al., 2008). Micronutrient deficiencies might fertility, embryogenesis and placentation and their prophylactic use in preventing these adverse pregnancy outcomes have been advocated (Cetin et al., 2009).

This study is therefore aimed at understanding the interactions of progesterone, heavy metals and micronutrients in influencing recurrent pregnancy loss in non-obese women in order to understand underlying mechanisms for both prevention and informed management.

MATERIALS AND METHODS

Subjects

A total of 90 apparently healthy women with normal body mass index (BMI) of 18.5-24.9 kg/m² (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 LH values, and normal transvaginal / abdominal scan done recently 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.

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 ethylenediamine 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 -20°C until analysis.

Demographic Indices

Semi structured pre-test questionnaire was completed by each subject in order to obtain demographic data. The demographic indices 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).

Anthropometric Indices

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

Biochemical Indices

The biochemical indices 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 phosphotungstic 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).

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.

RESULTS AND DISCUSSION

Results

Table 1 shows the comparison of demographic, anthropometric, lipids and progesterone between women with recurrent abortion (cases) and controls . Of all indices tested only NPP and TG were significantly higher while progesterone and NLB were significantly lower in cases compared with controls($p < 0.0001$).

Table 2 shows the comparison of micronutrients and heavy metals between women with recurrent abortion (cases) and controls. All micronutrients tested except Zn were significantly lower while heavy metals-Cd and Pb were significantly higher in cases compared with controls($p < 0.013$).

Table 3 shows correlations between progesterone and indices tested. Only age and weight showed positive correlations with progesterone in cases only($p < 0.026$). No correlation was observed between all indices tested and progesterone in controls ($p > 0.05$).

Discussion

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). In our study of non-obese women(aged < 35yrs with cases age matched with controls), progesterone levels were significantly lower in cases than controls($p=0.000$). Comparisons between cases and controls also showed significantly higher NPP and lower NLB in cases compared with controls($p=0.000$). These findings confirm earlier reports(Dhont,2003; Romero, 2007, Kroon et. al.,2011). 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).Cholesterol insufficiency impedes the initiation of sex hormones resulting in foetal loss (Shekerez-Bartho et. al., 1990). Our data show no significant differences in lipid fractions-TC, LDL and HDL($p>0.318$) between cases and controls. Correlations of progesterone with all lipid fractions tested were also not significant ($p>0.985$).

However, the finding of higher TG levels in cases than controls ($p=0.025$)in our study is contrary to an earlier study(Oladimeji et. al., 2002) in same geographical sub-region as ours, which reported similar levels of triglyceride in both women with RPL and controls. Metabolic syndrome characterised by increased levels of triglycerides, abdominal obesity and insulin resistance is now prevalent in Nigeria. 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). Recent studies demonstrated age and obesity as best predictors of foetal loss in the next pregnancy of women with RPL (Mostafa Metwally et. al.,2008, 2010; Kroon et. al.,2011). Correlations of progesterone with only age and weight observed in our cases only ($p<0.26$) emphasises their importance in RPL.

Chemical pollutants have been shown to interact additively to diet and lifestyle to modulate endocrine function. Cd and Pb are known to accumulate in fish and consumption of fish has been linked to adverse pregnancy outcomes(Farombi et. al.,2007, Carserta et. al.,2011). Cd and Pb are EDs that target the homeostasis of sex steroids and individuals could be exposed daily through multiple diverse pathways. Cd concentrates in the placenta of smokers and embryos(Thompson, Bannigan,2008; Caserta et. al.,2011). Endocrine disruptors and spontaneous abortion have significant association. Cd and Pb disrupt endocrine function and is a recognized risk factor for

Table1

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

184

Indices	Cases n = 60	Controls n = 30	T-test	p-value
Demographic Indices				
Age (years)	34.37±0.55	33.97±4.31	0.421	0.750
AM (years)	13.92±0.19	17.23±3.34	1.401	0.165
LMC (days)	28.45±0.40	28.77±1.48	0.553	0.596
NLB	01.43±0.12	02.67±0.14	6.123	0.000**
NPP	04.67±0.13	02.67±0.13	9.625	0.000**
Anthropometric Indices				

Height (m)	01.70±0.02	01.68±0.02	0.612	0.542	185
Weight (kg)	64.22±0.66	64.70±0.71	0.455	0.650	186
BMI (kg/m ²)	22.74±0.43	22.87±0.54	0.177	0.860	187
WC (cm)	16.93±0.26	16.93±0.31	0.000	1.000	188
HC (cm)	47.80±0.47	46.73±0.50	1.408	0.163	189
WHR	0.353±0.003	0.359±0.004	1.238	0.219	190
Lipids					191
TC (mg/dl)	139.15±5.00	153.50±3.80	1.895	0.061	192
TG (mg/dl)	93.15±3.41	90.67±5.38	2.288	0.025*	193
HDL(mg/dl)	45.30±0.64	46.40±0.89	1.004	0.318	194
LDL(mg/dl)	75.55±4.94	88.97±4.28	1.760	0.082	195
Progesterone(nmol/L)	04.84±0.61	18.35±3.09	5.768	0.000**	196

197

*= 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

Table 2
Comparison of Micronutrients and Heavy Metals between Women with Recurrent Abortion (cases) and controls

Micronutrients	Cases n= 60	Controls n= 30	T-test	p- value
Zinc (μmol/L)	13.45±0.80	12.24±0.43	1.026	0.308
Selenium (μmol/L)	08.46±0.21	11.95±0.36	8.862	0.000**
Manganese(μmol/L)	09.49±0.18	11.04±0.36	4.390	0.000**
Chromium (μmol/L)	11.14±0.21	12.21±0.41	2.556	0.012**
Magnesium (μmol/L)	00.75±0.04	01.04±0.03	4.931	0.000**
Iron (μmol/L)	11.42±0.67	18.36±0.77	6.312	0.000**
Vitamin E (mg/dl)	03.48±0.24	06.89±0.30	8.590	0.000**
Copper (μmol/L)	11.71±0.44	15.39±0.54	5.029	0.000**
Toxic trace metals				
Cadmium(μmol/L)	03.58±0.17	02.45±0.13	4.303	0.000**
Lead(μmol/L)	02.93±0.08	02.05±0.33	3.416	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

hormonal changes, impaired placental development, congenital malformations, fetal growth restriction and spontaneous abortion(Caserta et. al.,2011). Significantly higher levels of Cd and Pb in cases than controls($p<0.002$) were observed in this present study. Metal excretion has been correlated with progesterone and their negative impact on ovarian and pituitary function may be important in pregnancy loss(Gerhard et. al.,1998). However, Cd and Pb did not correlate with progesterone ($p>0.481$) in both cases and controls in our study. 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(Caserta et. al,2011).

Increased OS seems a common end-point to several endocrine disruptors(Caserta et. al.,2011). Female OS is 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

Table 3

Correlations of Progesterone with Demographic and Anthropometric Indices, Lipids, Micronutrients and Heavy metals in Women with Recurrent (Cases) and controls using Pearson's correlation

Biochemical indices	PROGESTERONE (nmol/L)
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	Cases n= 60		Controls n= 30	
	r-value	p-value	r-value	p-value
Age (years)	+0.290*	0.025*	+0.001	0.995
Age at menarche (years)	+0.052	0.696	-0.121	0.525
LMC (days)	-0.020	0.879	+0.169	0.371
NLB	+0.062	0.640	-0.011	0.952
NPP	+0.059	0.654	-0.072	0.704
Height (m)	+0.183	0.162	-0.280	0.135
Weight (kg)	+0.325	0.011*	-0.124	0.514
BMI (kg/m ²)	-0.138	0.294	+0.270	0.149
Waist circumference (cm)	-0.062	0.637	+0.118	0.535
Hip circumference (cm)	-0.174	0.184	-0.173	0.361
Waist Hip Ratio	+0.114	0.384	+0.176	0.352
Zinc (μmol/L)	-0.020	0.880	+0.058	0.759
Selenium (μmol/L)	+0.051	0.698	+0.281	0.133
Manganese (μmol/L)	-0.022	0.869	-0.195	0.303
Chromium (μmol/L)	+0.028	0.833	-0.273	0.144
Magnesium (μmol/L)	-0.011	0.934	-0.169	0.371
Iron (μmol/L)	-0.161	0.219	+0.176	0.352
Vitamin E (mg/dL)	+0.081	0.538	+0.194	0.303
Copper (μmol/L)	+0.041	0.757	-0.139	0.462
Cadmium (μmol/L)	-0.043	0.742	-0.045	0.814
Lead (μmol/L)	+0.150	0.912	-0.134	0.482
Total cholesterol (mg/dl)	-0.080	0.542	+0.054	0.778
Triglyceride (mg/dl)	-0.008	0.950	+0.095	0.619
High density lipoprotein (mg/dl)	-0.058	0.658	+0.003	0.985
Low density lipoprotein (mg/dl)	-0.046	0.725	+0.023	0.903

* = significant, n = number of subjects, r = Pearson's correlation, p = probability, LMC= Length of menstrual cycle, BMI=Body mass index, Cases= Non-pregnant women with history of recurrent abortion, controls= Non-pregnant women without history of recurrent abortion, NLB= Number of live births, NPP= Number of previous pregnancy

compartments. Retardation of trophoblastic outgrowth and development, placental necrosis and suppression of steroid 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).

Our data show that micronutrients(Fe, Cu, Mn, Mg, Cr, Se, Vit.E) were significantly lower in cases compared with controls ($p<0.013$). 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). However, no significant difference was demonstrated in Zn levels between cases and controls in our study($p=0.308$). This observation supports report of synergistic action of multinutrients to prevent adverse pregnancy outcomes(Haider et. al.,2011).

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). Rapid non-genomic events might critically determine the functional stimulation and/or response of/to progesterone in the female reproductive system in a cell-type- and environment-specific manner(Gellerson et. al., 2009). Although the overall effect of toxic metals on any cell or tissue is likely to be due to a synergism of several mechanisms, it is possible that one mechanism will predominate in a specific cell type(Thompson, Bannigan,2008). 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). 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). 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). Although sample size might have its limitation on our study, our study show the impact of the environment in women with RPL. Healthcare strategies show focus on reduction of environmental pollution and increase awareness on healthy diet for optimal periconceptional micronutrient requirements(Certin et. al.,2009,Caserta et. al.,2011).Tightly monitored clinical trials on the safety, efficacy and effective delivery of maternal micronutrient supplementation in women with RPL after adjusting for maternal education or gestational age at initiation of supplementation(Kawai et. al.,2011) are recommended.

CONCLUSION

Our findings suggest that in non-obese women with RPL, reduced levels of progesterone and micronutrients, and high levels of endocrine disruptors/toxic heavy metals and tryglycerides as well as increasing age and weight, might be independent risk factors of RPL but interact through

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 periconceptional micronutrient requirements.

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