

Full Length Research Paper

Toxicological aspects of geophagia in pregnancy: Putative molecular mechanism involving oxidative stress

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Received 12 January, 2014; Accepted 4 September, 2014

A number of studies have demonstrated that geophagia can be beneficial and harmful. However, toxicological aspects of this earth, soil, clay eating habit are not fully investigated. In addition, low levels of antioxidants have been observed in pre-eclamptic women. Still, the molecular mechanism implicated oxidative stress in geophagia inducing pre-eclampsia has not been elucidated. Despite its absorbent properties in detoxifying phytotoxin, lowering the incidence of nausea, natural clay still remains toxic for human consumption in normal and long term conditions. Our previous studies have shown the implication of oxidative stress in pre-eclampsia characterized by maternal hypertension. Biomarkers of oxidative stress such as lipid peroxides (LPO), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX) and 8-OHdG correlated with the extent of oxidative stress in pre-eclampsia. Fenton reaction induced by the presence of iron produces a more powerful reactive oxygen species (OH radical) which may contribute to the exacerbation of this status; while, clay eating is believed to protect pregnant women from pre-eclampsia by inhibiting iron uptake from gastrointestinal environment and so reducing the risk of production of OH radical during pregnancy where iron deficiency has been observed, it may be harmful however in iron overload conditions. We have demonstrated that cellular hepato-carcinoma can be indeed induced in rats being iron-loaded by ferrocene supplementation of their diet. Taking into account this possibility, we are postulating that during pre-eclamptic pregnancy, a number of factors including oxidative stress, activated macrophages and activation of xanthine oxidase (XO) can exacerbate this pregnancy complication. In addition, the availability of free iron from soil may as catalytic trace element involve in Haber-Weiss and Fenton reactions producing more ROS/RNS and especially OH radical. We are therefore suggesting that the subsequent activation of the nuclear factor NF- κ B by ROS/RNS generated from activated macrophages stimulates the induction of COX-2. This enzymatic induction leading to the expression of inflammatory cytokines could be one of the molecular mechanisms underlying pre-eclamptic conditions. Supplementation of zinc or eating clay containing zinc could overcome such pregnancy complication.

Key words: Geophagia, pregnancy, molecular mechanism, oxidative stress, Haber-Weiss and Fenton reactions, superoxide dismutase (SOD), glutathione peroxidase (GPX), OH radical, 8-hydroxyl deoxyguanosine (8-OHdG).

INTRODUCTION

According to Coreil et al. (2000), geophagia may be stimulated by dietary deficiencies and appears to be ubiquitous. An overview of potential benefits and negative consequences of geophagia has been outlined by Young (2008) who indicated the mechanism of useful micronutrients from soil such as iron, zinc or calcium and also that of poisonous elements; such as lead, mercury, etc. A body of information has been provided by a large number of studies (Lacey, 1990; Abrahams and Parsons, 1996; Njiru et al., 2011).

Iron and zinc for example obtained through geophagia or normal diet can lose their beneficial effect if they are absorbed by clay. Their uptake from gastro-intestinal tract can indeed cause iron and zinc deficiencies with subsequent consequences respectively on the erythropoiesis inducing a syndrome hypochromic anemia described by Prasad (1991a; 1991b) and various clinical manifestations such as short stature, delayed sexual maturity, hepatosplenomegaly and delayed bone age reported by Korman (1990).

Harmful effect of geophagia in pregnancy has been demonstrated in a large number of studies related to the microbial and parasite infections such as helminthes and the presence in the soil of heavy metals such as arsenic, lead and cadmium (Hunter, 1973; Horner et al., 1991; Corbett et al., 2003; Wigle et al., 2008; Al-Rmalli et al., 2010; Baidoo et al., 2010; Nyanza et al., 2014). One case of fatal soil peritonitis in an African women suffering from geophagia has been reported (Woywodt and Kiss, 2000). This peritonitis is due to the perforation of sigmoid colon. Frequent consumption of clay during pregnancy has been considered as habit rather than physiological need for the cessation of undesirable manifestations such as nausea, vomiting occurring in the first trimester of pregnancy (Horner et al., 1991).

Numerous studies have shown that pre-eclampsia characterized by a gestational hypertension associated with pathological edema, proteinuria, coagulation abnormalities, reduced utero-placental blood flow, and intrauterine growth restriction, is one of the maternal complications of pregnancy which can result in fetal or

infant death. Obesity has been indeed strongly associated with high risk of pre-eclampsia (Sibai et al., 1995). However, other physiopathological features such as insulin resistance, increased lipid availability, higher cholesterol and triglyceride levels may also involve in increased risk for early pregnancy loss (de Weerd et al., 2003). Shed membranes particules of leucocytes (Meziani et al., 2006), oxidative stress (Hubel, 1999), elevated plasma lipids (Lorentzen and Henriksen, 1998) and activated neutrophils (Clark et al., 1998) are among factors inducing in endothelial cell activation during pre-eclamptic pregnancy. Under these conditions, placenta is under oxidative stress with increased production of lipoperoxides and decreased antioxidant protection (Walsh, 1998; Wang and Walsh, 2001). The subsequent release of ROS/RNS, tumor necrosis and myeloperoxidase by neutrophils will lead into the smooth muscle inflammation in pre-eclampsia.

Despite its absorbent properties in detoxifying phytotoxin, lowering the incidence of nausea, natural clay still remains toxic for human consumption in normal and long term conditions. In the absence of evidence implicating clay minerals or micronutrients in pre-eclampsia process, we have postulated that geophagia could exacerbate this pregnancy complication through reactive oxygen/nitrogen species (ROS/RNS) generated from activated macrophages. In addition, the release of free iron from ferritin during inflammatory process producing cytokines could be the cause of the production of hydroxyl radical. Haber-Weiss and Fenton reactions involving iron as catalytic trace element do generate this OH radical (Halliwell and Gutteridge, 1990; Blakely et al., 1990).

Haber-Weiss reaction: $\text{H}_2\text{O}_2 + \text{O}_2^{\bullet-} \rightarrow \text{O}_2 + \bullet\text{OH} + \text{OH}^-$

Fenton-type chemistry: $(\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^3 + \bullet\text{OH} + \text{OH}^-)$

Natural clay contains iron among other minerals. Chronic feeding of iron has been associated indeed with an increased free radical generation in the colon and increase

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Abbreviations: CAT, Catalase; Fe, iron; FOX, ferrous xylenol orange; H₂O₂, hydrogen peroxide; SOD, superoxide dismutase; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GRx, glutathione reductase; TAS, total antioxidant status; Hb, haemoglobin; LOO, lipid radical; LOO[•], alkoxy radical; LOOH, lipid hydroperoxides; LPO, lipid peroxide; MDA, malondialdehyde; NADH, nicotinamide adenine dinucleotide reduced form; O₂^{•-}, superoxide anion; •OH, hydroxyl radical; 8-OHdG, 8-hydroxy-deoxyguanosine; ROS, reactive oxygen species; RNS, reactive nitrogen species; MSR, methionine sulphoxide reductase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ABTS, 2, 2'-azino-di-[3-ethylbenzthiazoline sulphonate]; ORAC, oxygen radical absorbance capacity; Cox-2, cyclo-oxygenase, Nf-kB, nuclear factor kappa B; HELLP, haemolysis, elevated liver enzymes, low platelet count; WHO, World Health Organization.

lipid peroxidation and thus constitute a risk factor for colorectal cancer (Lund et al., 2001).

As a transition element, ionic form of iron participates in one-electron transfer reactions and plays an important role as a prosthetic group in enzymes that catalyze redox reactions. Unfortunately this capacity enables iron to participate in the above mentioned reactions such as "Fenton reaction" generating more potent secondary ROS. The extent of $\cdot\text{OH}$ formation is largely determined by the availability and location of the metal ion catalyst. The availability of free Fe is controlled in physiological systems. Fe that is not incorporated into iron-utilizing proteins is rendered largely unavailable for participation in free-radical reactions by sequestration in storage (ferritin) or transport proteins (transferrin).

Other by-products of lipid peroxidation such as malondialdehyde (MDA) (Marnett, 1999) and M₁G deoxyribose resulting from the combination of MDA and deoxyribonucleoside (Marnett, 2000) together with 8-hydroxy-2'-deoxyguanosine (8-OHdG) a major DNA damage metabolite (Wong et al., 2003; Guetens et al., 2002; Shigenaga et al., 1994) by free radicals may also be involved in inflammatory process increasing NF- κ B activation which will enhance COX-2 induction. Increase activity of this enzyme will indeed affect the production of isoprostanes formed from the peroxidation of arachidonic acid (Liu, 1999).

The question of biological association of lipid peroxidation and pre-eclampsia has been discussed by Gupta et al., (2009). Placental oxidative stress is involved in the etiopathogenesis of pre-eclampsia as a result of ischemic reperfusion injury. Intermittent and inadequate placental perfusion in the spiral arteries as a result of repeated hypoxia/reoxygenation insult may indeed lead to the activation of xanthine oxidase which is a source of superoxide anion (Many, 2000; Raijmakers, 2004). This ischemic reperfusion injury constitutes a promoter of lipid peroxidation and endothelial cell dysfunction observed in pre-eclampsia.

Fortunately, in healthy subjects or non-pregnant women, the extent of oxidative stress is minimized by a strong defense line of antioxidants against ROS/RNS. While superoxide dismutase (SOD) converts two superoxide anions ($\text{O}_2^{\cdot-}$) into hydrogen peroxide (H_2O_2), catalase (CAT) and glutathione peroxidase (GPx) convert H_2O_2 to oxygen and water. Vitamins C, E and A remove the newly formed free radicals before they can initiate chain reactions. A repair cell structures damaged by free radical attack are done by DNA repair enzymes and methionine sulphoxide reductase (MSR).

Apart from vitamins A and C supplementation which showed 54% reduction in the risk of developing pre-eclampsia, other supplementation based on iron (Mahomed, 1998) and a mixture of iron and folate (Mahomed and Hytten, 1989), magnesium (Makrides and Crowther, 2001), fish oil (Makrides et al., 2006) did not

prevent this gestational hypertension.

In contrast, only calcium supplementation has reduced the incidence of both pre-eclampsia and hypertension (Villar et al., 2003). Taking together the above considerations, we suggest that the subsequent activation of the nuclear factor NF- κ B by ROS/RNS generated from activated macrophages and activation of xanthine oxidase stimulates the induction of COX-2. This enzymatic induction leading to the expression of inflammatory cytokines could be one of the molecular mechanism underlying pre-eclamptic conditions. Supplementation of zinc or eating clay containing zinc could overcome such pregnancy complication.

MATERIALS AND METHODS

Human subjects

A total of 117 women were enrolled in this study: 32 controls (non-pregnant), 42 pregnant non-eclamptic and 45 pregnant pre-eclamptic (30-34 week gestation).

Selection criteria

Inclusion criteria

Control: non-pregnant women (20-35 years old) without any sign of hypertension, diabetes or viral infection. They were recruited from student enrolled at an academic Institution (Medical University of Southern Africa)

Pregnant pre-eclamptic and non-eclamptic women were recruited from consulting room at tertiary hospital (George Mukhari Hospital). For pre-eclamptic group, the following symptoms have been observed and noted: headache, upper abdominal pain, visual disturbance, hypertension (high blood pressure, proteinuria). More importantly, the consumption of soil and the location has been also recorded.

Exclusion criteria

All subjects having infectious diseases such as HIV, Hepatitis were excluded from this study. In addition, control subjects did not have hypertension or renal diseases not suffering from any other diseases affecting oxidative stress parameters such as cardiovascular diseases, anemia, family hypercholesterolemia, diabetes and cancer. Pre-eclamptic pregnant women taking iron for anemia were excluded from this study.

Animal subjects

Ninety (90) pregnant Wistar albino (*rattus Norvegicus*) rats (of initial weight 200 ± 5 g) were fed (*ad libitum*) a standard chow diet (AIN-93G Formulation) according the following dietary regimen:

Group 1 (control group) did not receive any supplementation. Groups 2 and 3 (Fe gp2 and Fe gp3) were fed with a high iron diet (the diet was supplemented with 2.5% pentacarbonyl iron (CI-98.0% purity) according to the protocol of Plummer et al. (1997). Group 3 (Fe + vitamins C and E) received iron plus vitamins C and E supplementation: 1000 mg vitamin C per kg diet (ascorbic acid) and 500 mg vitamin E per kg diet \equiv 750 IU α -tocopherol (10x RDA)

(Plummer et al., 1997). At 12 month, the 2.5% CI was replaced by 0.5% dicyclopentadienyl iron [$\text{Fe}(\text{C}_5\text{H}_5)_2$] (CAS-102-54-5) (because of poor iron loading). The rats received human care in accordance with the guidelines of the Animal Ethics Committee of the University. They were studied for 24 months. Five rats from each group were sacrificed every four months up until 24 months for blood sampling. These samples were used for oxidative stress and transaminases evaluation.

Materials and chemicals

All chemicals, Vitamins and Kits for iron and ORAC evaluation were purchased from Sigma Company (Munich, Germany). For other tests, we used kits from Randox Laboratories, UK and Roche Diagnostics (Indianapolis, IN).

Analytical methods

Apart from selenium, vitamins A and E, all procedures using kits commercially available were followed according to the manufacturer's instructions.

i) Iron was evaluated by potentiostatic coulometry using a kit commercially available from Sigma Company. This method has been adapted by Dorner et al. (1981) to measure iron in small sample volume.

ii) Lipoperoxide (LPO) concentrations were measured spectrophotometrically at 560 nm using ferrous oxidation with xylenol orange (FOX II) assay according to the method of Nourooz-Zadeh et al. (1994) based on the principle of rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acidic conditions.

iii) 8 hydroxyl deoxy-guanosine (8-OHdG): Plasma levels of 8-OHdG were evaluated at 450 nm on a microplate plate reader using a commercial kit from the Japan Institute for the Control of Aging (Fukuroi, Japan). The method is based on a competitive *in-vitro* enzyme-linked immunosorbent assay for quantitative measurement of this DNA metabolite in tissue, serum and plasma (Toyokuni et al., 1997 and 1999).

iv) Superoxide dismutase (SOD): Erythrocyte SOD activity was determined by the method of Arthur and Boyne (1985) using a commercial kit obtained from Randox Laboratories, UK. This method uses xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity is then measured spectrophotometrically at 505 nm by the degree of inhibition of this reaction.

v) Glutathione peroxidase (GPx): The determination of erythrocyte GPx activity was based on modification of the method of Paglia and Valentine (1967) using a commercial kit obtained from Randox Laboratories, UK. This method involves the oxidation of glutathione (GSH) by cumene hydroperoxide catalyzed by GPx. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP^+ and the decrease in absorbance is then measured spectrophotometrically at 340 nm.

vi) Transaminases: Aspartate aminotransferase (AST) and alanine aminotranferase (ALT) activities were evaluated using an automate auto-analyser (Cobas Interga 400, Holliston, MA) and kits from Roche Diagnostics (Indianapolis, IN), using the spectrophotometric method of Karmen (1955) in which malic acid dehydrogenase is added to convert the oxaloacetic acid formed by AST to malic acid with the simultaneous oxidation of the coenzyme NADH_2 (reduced nicotinamide adenine dinucleotide) to NAD (nicotinamideadenine dinucleotide).

In the case of ALT, lactic acid dehydrogenase is used and lactic acid is formed simultaneously with NAD. The decrease in absorbance of NADH_2 as it is oxidized to NAD is followed at 340 nm by the spectrophotometer in each case.

vii) Oxygen radical absorbance capacity (ORAC): Liver total antioxidants were measured using oxygen radical absorbance capacity (ORAC) system which is made up of B-phytoerythrin as a fluorescent indicator protein, 2,2'-azo-bis(2-aminodipropyl) dihydrochloride a peroxy radical generator, and the water-soluble vitamin E analogue Trolox (Aldrich-Sigma, Munich, Germany) as a reference standard. The total antioxidant capacity was expressed as ORAC units, where one unit equals the net protection by 1 μmol Trolox/l (Cao and Prior, 1999).

viii) Total antioxidant status (TAS): Total antioxidant status (TAS) in heparinised plasma was determined according to Miller et al. (1993) method using a commercial kit obtained from Randox Laboratories Limited, U.K. In this method 2, 2'-azino-di-[3-ethylbenzthiazoline sulphinate] (ABTS^{R}) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $\text{ABTS}^{\text{R}+}$ measured at 600 nm.

ix) Selenium: Selenium in plasma was estimated by the method of Pleban et al. (1982).

x) Vitamin C (ascorbic acid) was estimated spectrophotometrically at wavelength of 700 nm in plasma by acid phosphotungstate method of Aye (1978).

xi) Vitamin E (α -tocopherol): Vitamin E (α -tocopherol) in plasma was extracted using xylene, and its level was estimated spectrophotometrically at 520 nm according to the method of Baker and Frank (1968).

Statistical analyses

All data were presented as mean \pm standard deviation (SD) for each biomarker. The SAS statistical package was used. Statistical analysis was performed by independent Student's *t*-test. A probability value of $p < 0.05$ was considered statistically significant.

RESULTS

Experimental animals

The data obtained from pregnant Wistar albino (*rattus Norvegicus*) rats fed high iron diet with and without vitamin supplementation demonstrated that vit C and E reduced for a limited period of time (20 months) the extent of oxidative stress as shown by the increased activity of SOD and GPx with subsequent decrease of LPO and 8-OHdG levels (Table 2). Hepatic enzymatic activities of transferases ALT and AST are also increased respectively by 29 and 34%. It has been noted that over a prolonged period, supplementation of high doses of vitamins C and E did not reduce the adverse effect of ROS/RNS and the by-products of lipid peroxidation and attenuate the subsequent oxidative DNA damage leading to cancer.

DISCUSSION

Several trials failed to establish links between pre-eclampsia

Table 1. Biomarkers of oxidative stress in pregnancy and pre-eclampsia in humans

Parameter	Control	Normal pregnant women	Pre-eclamptic women
LPO ($\mu\text{mol/L}$)	32.71 \pm 5.84	55.45 \pm 9.07	68.19 \pm 6.44
8-OHdG (ng/ml)	33.52 \pm 4.61	48.34 \pm 8.45	50.24 \pm 5.96
SOD (U/g Hb)	4394.51 \pm 1152.33	4134.12 \pm 532.67	4005 \pm 1037.28
GPx (U/g Hb)	1173.49 \pm 234.75	970.64 \pm 145.34	
TAS (mmol/L)	1.45 \pm 0.18	1.23 \pm 0.12	0.62 \pm 0.14
Vitamin C(mg/dl)	0.12 \pm 0.06	0.10 \pm 0.05	0.07 \pm 0.03
Vitamin E (mg/dl)	1.13 \pm 0.07	1.09 \pm 0.35	0.95 \pm 0.24
Selenium ($\mu\text{mol/L}$)	0.85 \pm 0.24	0.76 \pm 0.20	0.38 \pm 0.15

Table 2. Biomarkers of Oxidative stress in iron overloaded animal (rats0 model at 20 month

Group	Fe ($\mu\text{mol/L}$)	ALT (IU/L)	AST (IU/L)	SOD (U/g Hb)	GPX (U/gHb)	ORAC(mM Trolox)	8-OHdG (nmol/ml)
Group 1 (control)	62.5 \pm 6.2	63.2 \pm 5.3	130 \pm 4.6	2165 \pm 444	214 \pm 11.9	14.2 \pm 2.8	123 \pm 21
Group 2 (Fe)	122.0 \pm 10.5	329 \pm 48.6	202.2 \pm 50.5	1584 \pm 360	179.1 \pm 16.1	7.5 \pm 1.4	455 \pm 151
Group 3 (Fe + Vit C & E Supplementation)	119.1 \pm 9.2	234.6 \pm 48.3	134.6 \pm 48.6	1626 \pm 199	228.8 \pm 17.6	13.0 \pm 2.5	346 \pm 117

and the following interventions: balanced protein-energy supplementation (Kramer, 2000), isocaloric balance protein supplementation (Kramer and Kakuma, 2003), and protein and energy restriction for obese women (Kramer, 2007) despite the relationship between pre-eclampsia and obesity.

It has been reported that calcium reduce the risk of gestational hypertension (Villar et al., 2003) in contrast with the non-significant effect of calcium supplementation observed in the largest trial conducted by the National Institute of Health-USA. These negative observations have been made for iron and folate supplementation as above mentioned. There is no biological link either between magnesium, fish oil and gestational hypertension (Altman et al., 2002; Duley, 2009).

Fortunately, prophylactic interventions with vitamins E and C supplementation were associated with 54% reduction in the risk of developing pre-eclampsia. Among various WHO recommendations (WHO, 2011a; 2011b) for prevention and treatment of pre-eclampsia and eclampsia, the high quality of evidence has been observed only for magnesium sulfate (Duley et al., 2010a, 2010b), vitamins C and E administration (Rumbold et al., 2008) which make them to be strongly recommended (WHO, 2010, 2011). Calcium (Hofmeyr et al., 2010) and vit D supplementation and corticoids administration especially for the treatment of the "haemolysis elevated liver enzymes low platelet count" (HELLP) can help (Woudstra et al., 2010). However these supplementation programmes should be monitored and evaluated for their effects.

Usually labour induction and administration of antihypertensive drugs (Duley et al., 2006; Abalos et al., 2007) and diuretics (Churchill et al., 2007) for pre-eclampsia or gestational hypertension at term are recommended. However, the benefit and potential harm of these strategies need to be investigated especially in rural African settings where accurate gestational age assessment is difficult to be conducted due to the late initiation or absence of antenatal care.

Data from our studies, confirmed those reported by Gupta et al., (2009) on lipid peroxides and antioxidants status in pre-eclampsia. As shown in Table 1, biomarkers of oxidative stress (LPO, 8-OHdG) increased while enzymatic activities (SOD, GPx) decreased more during pre-eclampsia than the normal pregnancy suggesting the involvement of ROS/RNS from the activation of macrophages.

The decrease of GPx activity, a seleno-enzyme, observed in iron overload rats (Table 2), may justify the reduction of this micronutrient: selenium being used in biosynthesis of this enzyme in humans and animals.

In contrast, TAS, vitamins C and E were reduced suggesting their role in scavenging free radicals and especially OH radical generated from Haber-Weiss and Fenton reactions and from the activation of macrophages. Those reactions take place because of the release of free iron from ferritin during inflammatory process producing cytokines (Casanueva and Viteri, 2003). Excess iron intake through geophagia could induce toxic reactions (Rush, 2000). The resulting high ferritin level will lead into the failure of maternal plasma

expansion (Silver et al., 1998).

Our results have confirmed those of Scholl (2004, unpublished data) on the association between increased iron stores and the excretion of 8-OHdG, a marker of oxidative damage to DNA and may influence the long-term health outcomes of infants after birth by inducing G→T mutations during DNA replication which increase the risk of cancer (Satoko et al., 2012; Shibutani et al., 1991).

Our experimental animal model has indeed demonstrated in iron overload conditions, a decrease of total antioxidants and an increase of 8-OHdG in plasma as well as in rat hepatocytes; antioxidative enzymatic activities SOD and GPx being reduced by 26.9 and 16.4%, respectively. Supplementation in vit C and E can help to reduce the extent of oxidative stress: 2% for SOD and 27% for GPx. It can also alleviate maternal plasma expansion. Inadequate plasma volume expansion as depicted by the reduction of creatinin, uric acid and hematocrit has been indeed observed in women with pre-eclampsia (Silver et al., 1998).

However, high doses of vitamins ((Cheng, 1999; Muller, 2010) and antioxidants (Bjelakovic et al., 2007) supplementations can increase the mortality rate in randomized trials. Generally, food and to lesser extent, soil contains less heme iron than nonheme iron. The lower absorption rate iron containing soil may be increased depending on the level of iron stores (Monsen, 1988). Iron deficiency observed in many African pregnant women may thus trigger the absorption rate of this form of heme. Iron and zinc deficiencies usually associated with plant-based diets in poor countries are not associated with vegetarian diets in wealthier countries (Janet, 2003). The question of bioavailability of iron, zinc, and other trace minerals in pregnant women is not completely resolved and depends on factors such as gastric acidity, presence of inhibitors (Hurrell et al., 1999; Hunt, 2003). The relative bioavailability of iron compounds is determined by their solubility in stomach's gastric juice. Iron deficiency can be prevented using food fortification by addition of enhancers such as ethylen-diamine-tetraacetic acid, glycine, ascorbic acid (Hurrell, 1997). Hilt et al. (2011) have even demonstrated the improvement of iron solubility in dilute acid when Magnesium and Calcium have been incorporated into the nanostructure of Fe₂O₃. Nevertheless, in acidic and decrease of iron stores conditions, and in absence of inhibitors, nonheme iron can thus play a role as catalyst metal in Haber-Weiss and Fenton reaction producing more OH radical which will damage cells.

While the above mentioned clay minerals do not have apparent toxicity, iron may be harmful in pre-eclamptic/eclamptic women because of generation of ROS/RNS inducing through NF- κ B. the expression of inflammatory genes. This gene expression is susceptible to exacerbate the gestational hypertension in

short term and cancer in long term process; inflammation being the intermediate step before the occurrence of pre-neoplastic phase.

In contrast, zinc supplementation needs to be taken into consideration because of its role in the metabolism of several protein enzymes. Deficiency in this important trace element can indeed affect many functions including the induction of pre-eclampsia. It also plays an important role in cell-mediated immune functions as anti-inflammatory and antioxidant agent (Prasad, 2004).

Conclusion

Due to its ubiquitous properties, eating soil containing iron may be harmful for pregnant and mostly pre-eclamptic/eclamptic women where endothelial dysfunction can occur as a result of activation of macrophages. As molecular mechanism, ROS/RNS resulting from activated macrophages will trigger the binding of AP-1 to NF- κ B for the induction of COX-2 leading to the expression of inflammatory genes responsible for clinical manifestations of pre-eclampsia.

Soil containing magnesium and zinc should be beneficial to pregnant as well as pre-eclamptic/eclamptic women. In the particular case of zinc, this trace element will stimulate the upregulation of mRNA and DNA-specific binding for A20. This transactivating factor will then inhibit the activation of NF- κ B leading to the down regulation of inflammatory cytokines (Prasad et al., 2004, 2011; Bao et al., 2010).

Conflict of Interests

The author(s) have not declared any conflict of interests.

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