Levels of Micronutrients and Heavy Metals in Cord Blood and Maternal Blood of Electric Stove Users Compared With Firewood Users.

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Abstract

Introduction

Exposure to smoke from biomass fuel causes chronic diseases, but the mechanism underlying negative effects of household air pollution (HAP) is not exactly understood. The basis of the impact of HAP on maternal and fetal health was assessed by determining the levels of teratogenic heavy metals [Lead (Pb), Mercury (Hg)] and micronutrients associated with DNA methylation [Zinc (Zn), Iodine (I), vitamins B6 and B12, folic acid and homocysteine] in cord blood of babies and maternal blood of electric stove users compared with firewood users. Levels of Pb, Hg and Zn were determined by atomic absorption spectrophotometry (AAS). Folic acid, homocysteine, vitamins B6 and B12 were measured using enzyme link immunosorbent assay (ELISA).

Zn level was significantly higher whereas Pb level was significantly lower in sera of mothers using electric stoves compared with mothers using firewood. There were no significant differences in the levels of micronutrients and heavy metals in cord sera of babies born by mothers using electric stoves compared with the levels of micronutrients and heavy metals in cord sera from cord sera of babies born by mothers using firewood. Pb in maternal sera showed significant positive correlation with Pb in cord sera of babies born by mothers using firewood whereas vitamin B12 in maternal sera showed significant positive correlation with vitamin B12 in cord sera of babies born by mothers using electric stove.

Significantly high Pb level coupled with low Zn level in mothers using firewood might be responsible for adverse effects of firewood on the users. Our study therefore recommends Zn supplementation for those exposed to HAP from firewood. Thus, global DNA methylation is proposed as further study to explain the effects of HAP.

Key words: Indoor Pollutants, DNA Methylation, Mothers, Neonates, Micronutrients supplementation.

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Indoor air pollution can be traced to prehistoric times when human first used fire for cooking, warmth and light¹. Approximately half of the world's population and up to 90% of rural households in developing countries rely on unprocessed biomass fuels in the form of fire-wood for domestic uses². Soot from wood combustion in poorly ventilated places produce high levels of indoor pollution^{3, 4} containing sulfur oxides, nitrogen oxides, carbon monoxide and potentially carcinogenic compounds (polycyclic aromatic hydrocarbons PHAs, CO₂, NO, benzene, formaldehyde, dioxins and free radicals)⁵ which are known to cause cancers, cataracts, pulmonary diseases⁶, increased risk of stillbirth, low birth weight and impaired cognitive development^{7,8}. Modernization and improved socio-economic status have been accompanied by a shift from biomass fuels such as wood to petroleum products (such as kerosene and gas) and electric stove9, 10. However, most households in Nigeria still use firewood because of its relative cheapness and availability. Other households combine the uses of kerosene stoves with firewood, gas stoves and electric stoves depending on economic situations¹¹. Mothers and babies during cooking are mostly exposed to fine particulate matters from indoor air pollutants⁹.

Mechanism by which the constituents of HAP initiates or propagates the harmful effects is not fully understood but one of our previous studies suggested induction of oxidative stress and inflammation⁴. Some groups have also shown that both oxidative stress and nitric oxide can affect DNA methylation status³¹. Nutrition is known to play an important role in DNA methylation, as many dietary micronutrients such as folate, vit. B12 and Zn are directly involved in DNA methylation pathways though some of these micronutrients are antagonized by heavy metals such as Pb and Hg³². Micronutrients are also important in determining genomic stability as it affects all relevant developmental pathways such as activation/ detoxification of chemicals preventing DNA oxidation, DNA repair, apoptosis and DNA synthesis³³. Folate and vitamin B12 deficiencies cause DNA hypomethylation, a defect that leads to chromosome $loss^{33}$.

Previous studies have reported increased levels of heavy metals such as Pb and Cd in animal products processed with firewood¹² and analysis of ashes from burnt firewood revealed significantly high Pb level¹². Heavy metals have been implicated to be antagonistic to the availability and functions of essential micronutrients³². Thus, it is likely that chronic exposure to components of HAP will affect micronutrients associated with DNA methylation in the users of firewood. This hypothesis is reinforced by the fact that HAP from firewood contains heavy metals, induces oxidative stress, regulates DNA methylation and increases susceptibility to chronic diseases. The outcome of this study will predict future effects of firewood smoke on babies born to the users, thus may lead to recommendation of cleaner mode of cooking or recommend use of supplements that contains micronutrients which modulates DNA methylation to those that must use biomass fuels.

Despite significant negative health effects of exposure to HAP, less information is available to explain these observations. The study therefore determined micronutrients that modulate DNA methylation (Zn, I, vit B6, vit B12, folate and homocysteine) and some heavy metals (Pb and Hg) in cord blood of babies and mothers using firewood compared those using electric stoves.

Materials and method Study Site and Subjects

A cross sectional study was carried out to determine the micronutrient and heavy metal status of pregnant mothers and cord blood of babies born by women who engaged in the use of fire-wood or electric stoves for cooking. The subjects were selected from Moniya Maternity Centre and University College Hospital, Ibadan, Nigeria. Out of 229 women recruited, only six of them cooked solely with firewood and six cooked solely with electric stove for at least 10 years were included. Questionnaire was administered to determine dietary pattern to exclude those with health defects that could impair

 Table 1: Mean serum levels of micronutrients and heavy metals in women using electric stoves compared with those using firewood.

micronutrient status and those on any supplement or compulsory medication. Also excluded were those with pregnancy complications, with any infectious microorganisms and those using other cooking methods apart from fire-wood and electric stoves. Ethical consideration to carry out the study was obtained from Oyo State Ministry of Health, Nigeria. All the pregnant mothers consented to participation in the study.

Blood, urine and faecal samples were collected by trained personnel and were processed as previously described to exclude those with any infectious agent¹³, ¹⁴. Serum levels of Pb, Hg and Zn were determined using Atomic absorption spectrophotometry (AAS). Homocysteine, vitamins B6 and B12 were measured using Enzyme link immunosorbent assay (ELISA) as previously described¹⁵. The quantification of iodine was performed by a potentiometric method using an Iodide ISE¹⁶.

Results

Zn level was significantly higher whereas Pb level was significantly lower in sera of mothers using electric stoves compared with mothers using firewood (Table 1). There were no significant differences in the levels of micronutrients and heavy metals in cord sera of babies born by mothers using electric stoves compared with the levels of micronutrients and heavy metals in cord sera of babies born by mothers using firewood (Table 2). The correlations between levels of micronutrients or heavy metals in maternal sera compared with levels of micronutrients or heavy metals in cord sera were presented in Table 3 and 4. Pb in maternal sera showed significant positive correlation with Pb in cord sera of babies born by mothers using firewood (Table 4) whereas vitamin B12 in maternal sera showed significant positive correlation with vitamin B12 in cord sera of babies born by mothers using electric stove users (Table 3).

Table 2: Mean cord serum levels of micronutrients and heavy metals
in women using electric stoves compared with those using firewood.

women using electric stoves compared with those using firewood.				in women using electri	c stoves compare	d with those using	g firewood
Parameters	Electric stoves (n=6)	Firewood (n=6)	p-value	Parameters	Electric stoves	Firewood	p-value
Zn (µg/dl)	99.09±5.3	81.18±6.2	0.019*	$Zn (\mu g/dl)$	124.70±12.3	121.84±14.4	0.807
Pb (µg/dl)	3.04±0.5	6.41±1.0	0.046*	Pb (µg/dl)	4.15±1.3	4.29±1.4	0.900
Hg (μ g/L)	9.17±0.9	9.23±0.5	0.917	Hg (μ g/L)	10.57±0.5	11.43±2.3	0.565
I (μ g/L)	54.45±6.9	49.88±10.4	0.561	I (μ g/L)	64.76±3.3	69.95±14.4	0.576
Vit B6 (pg/mol)	6.98±1.45	7.19±0.5	0.825	Vit B6 (pg/ml)	8.37±1.7	7.49±1.7	0.559
Vit B12 (pg/mol)	441.49±72.0	401.01±42.1	0.448	Vit B12 (pg/ml)	372.37±25.2	322.66±43.5	0.162
Folic acid (ng/ml)	9.55±2.7	9.96±0.6	0.807	Folic acid (ng/ml)	10.42±2.7	10.19±1.4	0.899
Homocysteine (µmol/	L) 7.78±1.6	7.68±0.5	0.924	Homocysteine (µmol/L)) 10.44±1.0	10.09±2.1	0.807
*Showed significant	nt differences						

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Table 3: Correlation of levels of micronutrients and heavy metals in cord sera with the levels in sera of mothers using electric stoves

		r-value	p-value
Electric	Zinc (Maternal) & Zinc (Cord blood)	828	.379
	Pb (Maternal) &Pb (Cord blood)	946	.211
	Hg (Maternal) & Hg (Cord blood)	.640	.558
	Iodine (Maternal) & Iodine (Cord blood)	.286	.815
	Vit B6 (Maternal &Vit B6 (Cord blood)	.759	.452
	Vit B12 (Maternal) &Vit B12 (Cord blood)	1.000	.009*
	Folic Acid (Maternal) & Folic Acid (Cord blood)	.985	.112
	Homocysteine (Maternal) & Homocysteine (Cord	.965	.168
	blood)		

*Showed significant difference

Table 4: Correlation of levels of micronutrients and heavy metals in cord sera with the levels in sera of mothers using firewood.

		r-value	p-value
Firewood	Zinc (Maternal) & Zinc (Cord blood)	.091	.942
	Pb (Maternal) & Pb (Cord blood)	1.000	.006*
	Hg (Maternal) & Hg (Cord blood)	985	.109
	Iodine (Maternal) & Iodine (Cord blood)	.122	.922
	Vit B6 (Maternal &Vit B6 (Cord blood)	.979	.132
	Vit B12 (Maternal) &Vit B12 (Cord blood)	.693	.513
	Folic Acid (Maternal) & Folic Acid (Cord blood)	.664	.538
	Homocysteine (Maternal) & Homocysteine (Cord	.985	.110
	blood)		

*Showed significant difference

Discussion

Exposure to HAP from biomass smoke has been implicated in inflammation and in the development of respiratory diseases^{17, 18}. Also, studies have linked significant HAP exposure of pregnant women with increased adverse effects in foetus¹⁹. Although the underlying mechanisms for HAP-related injury are still being investigated, exposure inducedoxidative damage has been alleged to play an important role^{4,20}.

In the present study, mean serum level of Pb in pregnant women using firewood was significantly higher compared with mothers using electric stove. This is an indication that Pb from firewood smoke might have entered the blood circulation of women using firewood. A previous study reported accumulation of Pb in firewood smoke^{12, 22}. The present study also observed significant positive correlation of Pb in maternal and cord blood of firewood users. Thus, supporting the fact that Pb in the bloodstream of mothers might have been transferred to the fetus. Our result corroborates a previous finding that Pb readily crosses the placenta by passive diffusion and has been detected in the fetal brain as early as the end of the first trimester²³. Elevated Pb levels in pregnancy have been associated with gestational hypertension, spontaneous

abortion, low birth weight, and impaired neurodevelopment²⁴. Epidemiological and toxicological studies showed that epigenetic alterations induced by Pb may be the key mechanisms linking Pb exposure to its toxicity^{25, 26}. This is in line with a report that Pb induces oxidative stress causing damage to membrane, DNA and proteins. Therefore, increased level of Pb as observed in the present study might induce epigenetic changes and oxidative stress, thus the reason for the adverse effects of Pb in firewood users.

Our present study showed significantly reduced Zn level coupled with raised level of Pb in mothers using firewood. Zn and Pb compete for similar binding sites on the metallothionein-like transport protein in the gastrointestinal tract. Excessive inhalation or indirect ingestion of Pb from firewood smoke might have caused decreased absorption of Zn thus reducing blood Zn level in firewood users. Dietary supplementation with Zn and in combination with ascorbic acid and thiamine was shown to reduce Pb toxicity in humans²⁶. Thus, the protective effects of zinc against oxidative damage may support its recommendation for mothers who must use firewood.

There are data suggesting Pb-induced alteration in gene expression by interacting with zincbinding sites on DNA-associated <u>protein</u> (protamine). Zn has a relationship with many enzymes and prevents cell damage through activation of the antioxidant system²⁷. Zn deficiency has been shown to increase lipid peroxidation in rat tissues and Zn has also been reported as an essential component of the antioxidant defense system²⁸. Thus, reduced Zn level coupled with raised level of Pb in mothers using firewood further support oxidative stress hypothesis as a basis of HAP-induced adverse effects in mothers that use firewood.

Cord blood analysis has been reported to provide individual measure of prenatal exposure to HAP. Prenatal exposure to pollutants in HAP has been shown to increase chances of neuro-developmental disorders²⁹, which may be modified by micronutrients. In this study, vitamin B12 in maternal blood showed significant positive correlation with cord blood vitamin B12 in electric stove users. This implies adequate transfer of vitamin B12 from mothers to fetuses in utero. A research showed that adequate intake of vitamin B12 and folate in pregnancy prevents neural tube defects such as spinal bifida, low cognition as well as megaloblastic anaemia in babies³⁰. Therefore, women that use electric stove might have better pregnancy outcomes than firewood users. However, in the firewood users, the level of Pb in the mothers showed significant positive correlation with cord Pb level. This suggests potential adverse effects of Pb in the babies born to mothers exposed to HAP from firewood smoke.

Conclusion:

Significantly high Pb level coupled with low Zn level in mothers using firewood compared with mothers using electric stoves might be responsible for adverse effects of firewood on the users.

Limitation:

Low number of selected participants is due to strict adherence to selection criterion. Most women in Nigeria domestically use either kerosene stove, gas stove or combination of the two methods of cooking.

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