Effect of Kaolin Consumption on Serum Heavy Metal Levels of Pregnant Women

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Abstract: Geophagy is the consumption of earth materials across several cultures in many continents. For various reasons, consumption of kaolin is common among pregnant women in Nigeria. This earth material is known to contain heavy metals. This study was carried out to determine the effect of kaolin consumption on some heavy metal levels in pregnant women attending antenatal clinic in a Teaching Hospital in the south east Nigeria. A total of eighty pregnant women recruited for this study were grouped as follows; Group 1, (30 geophagic pregnant women within the first trimester). Group 2 (30 geophagic pregnant women within the second trimester) and Group 3 (20 non-geophagic pregnant women that served as control). Subjects in Groups 1 and 2 were subdivided into three based on the quantity of kaolin consumed per week. Group A (100 g/week), group B (150 g/week) and group C (200 g/week). Preliminary analysis of the heavy metal contents of kaolin was carried out. After two weeks of kaolin consumption, the serum levels of lead, arsenic, cadmium and mercury of the subjects were determined. The mean concentrations of lead (0.347 ± 0.12), arsenic (0.354 ± 0.15), cadmium (0.209 ± 0.11) and mercury (0.029 ± 0.02) in the kaolin exceeded the maximum permissible concentrations in food. The concentration of lead increased significantly (p < 0.05) in the first and second trimester pregnant women on 150 and 200 g of kaolin per week. There was a significant (p < 0.05) increase in the concentration of arsenic and cadmium in the second trimester women who consumed 200 g of kaolin per week. These results indicate that unregulated consumption of kaolin in pregnancy increases the serum level of the heavy metals and may be a risk factor for lead toxicity with its attendant complications in the infants.

Keywords: Geophagy, Kaolin, Pregnancy, Heavy Metals, Infants

Introduction
Geophagy is described as deliberate eating of soil (1), or intentional consumption of clay soil (2). Soil eating is common among child bearing and breastfeeding women in sub-Saharan Africa (3) and it cuts across socio-economic, ethnic, religious and racial divides (4).

There is increased nutritional demand during pregnancy, especially minerals such as iron and calcium, to support both the growing fetus and elevated blood production. This period of nutritional needs often occurs with the attendant digestive difficulties such as nausea and vomiting, generally referred to as morning sickness. This and the need to reduce hypocalcaemia-induced cramp and tetany (5) often enhance eating of kaolin during the first and second trimesters as anti-nausea agent (6).

Kaolin is a broad name given to a range of clay-compounds made of predominantly kaolinite and several other minerals produced by alteration of felspathic rocks. Kaolinite is composed of tiny sheets of triclinic crystals with pseudohexagonal morphology. Kaolin may be white, yellow, grey, pink, or red and has soft plastic nature (7).

Kaolin adsorbs heavy metals either via cation exchange in the inner layer resulting from interactions between negative charge ions or through the formation of inner sphere complexes at the particle edges (8). In 2002, the European Union alerted the Cameroon ministry of public health that kaolin from Cameroon had an abnormally high amount of lead at levels a 100 times higher than the permissible level (9).

Reports have largely disregarded the hypothesis that geophagy is a physiological response to nutrients, such as iron and calcium deficiencies (10). There are postulations that it rather creates than corrects, iron, zinc, or potassium deficiencies (11). Those who eat clay at the expense of healthier foods often develop malnutrition. Kaolin consumption during pregnancy is a common practice hence, there may be consequences for the unborn child (12).

In the first trimester of pregnancy, the foetus is particularly vulnerable to heavy metal toxicity. The pregnant mother is also vulnerable due to suppressed immune system (13). Heavy metals are considered one of the most important groups of environmental pollutants, which in small quantities are essential...
Nutrients for protecting health, but in greater quantity they can be toxic and dangerous (14). Heavy metals such as copper, arsenic, cadmium, lead and mercury have adverse effects on pregnancy. These metals are not filtered by placenta hence; they are carried from mother to child and are directly deposited in growing foetal tissue. This study evaluated the level of some heavy metals in the blood of pregnant women that consumed kaolin.

Nutrition during pregnancy is increasingly demanding. In hot and humid environment, kaolin develops negative charges between its layers and attracts positive charges (such as heavy metals) resulting in contamination (15). The use of kaolin to reduce metal bioavailability and resulting toxicity in underground water and sub soils has been successful for remediation of heavy metals polluted environment (16).

This phenomenon of nutrient deficiency is exacerbated when clays with high cation-exchange capacities are ingested. Complications from geophagia include hypokalemia, hypogonadism, and iron deficiency (17). Exposure to these toxic substances can be gotten from prolonged consumption of contaminated kaolin. These metals cause various degrees of damage in pregnancy (14).

Arsenic affects pregnant women who are at an increased risk for gestational diabetes. Chronic arsenic exposure through drinking water can increase fetal morbidity and mortality. Exposure to lead during pregnancy can cause miscarriage or premature birth with low birth-weight, and is often associated with brain developmental defects and mental retardation (18). Infants and fetuses exposed to lead can develop behavioral and learning disabilities. Mercury is transferred from the mother to the fetus via the placenta and to the infant through the breastfeeding. In the United States, it is estimated that one in ten fetuses are born annually with increased risk of neurological disorders due to mercury exposure during pregnancy. The mercury toxicity can damage the fetal developing nervous system causing learning difficulties and can affect the reproductive system, involving in problems such as infertility, miscarriage and premature birth (19). Various heavy metals such as lead, mercury and cadmium are known to alter the delicate balance of mother - fetus, potentially causing long - term damage in newborns. The thickness of placental layers is one of the major determining factors which affect the ability and interactive substance transport from the mother to the fetus (20).

Materials and Methods

Research design

This is a cross-sectional study involving eighty (80) pregnant women recruited from the antenatal clinic of Chukwuemeka Odimegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State, Nigeria. They were pregnant women within first and second trimester of pregnancy. The kaolin samples were obtained from the main market in Awka metropolis.

Ethical approval for the study was obtained from the Ethical Committee of the COOUTH (COOUTH/CMAC/ETH/C/VOL.1/01Q4 Date: 15/10/2018) before the commencement of the study, and written informed consent was obtained from each of the participants.

Methods

The subjects were divided into three main groups as follows: Groups 1 (30 geophagic pregnant women within the first trimester), Group 2 (30 geophagic pregnant women within the second trimester) and Group 3 (20 non-geophagic pregnant women that served as control). Groups 1 and 2 were subdivided into three (A-C) each (n=10) according to the quantity of kaolin consumed. The groups were treated as follows: Group A (14.3 g of kaolin/day), Group B (21.4 g of kaolin/day) and Group C (28.6 g of kaolin/day), equivalent to 100, 150 and 200 g of kaolin/week respectively.

Preparation of kaolin for heavy metal analysis

Dried kaolin (5 g) was placed into a digestion flask and 20 ml of acid mixture (650 ml conc HNO₃, 80 ml perchloric acid and 20 ml conc H₂SO₄) was added. The flask was then, heated until a clear digest was obtained. The digest was diluted with distilled water to 100 ml mark. Metals were measured using Atomic Absorption Spectroscopy (FS240AA, Agilent Technologies, South San Francisco, USA) (21).

Blood samples collection and preparation

Blood samples were collected from the subjects via venipuncture and dispensed into plain test tubes. The samples were allowed to clot, suspended and then centrifuged at 2500 g for 10 minutes. The serum was allowed to clot, suspended and then centrifuged at 2500 g for 10 minutes. The serum was separated from the cells and was kept in the freezer till they were ready for analysis.

Determination of heavy metals in kaolin

The heavy metals lead, mercury, cadmium and arsenic in kaolin were analyzed using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA (22). Stock solution containing 1000 parts per million (ppm) was prepared for each element. The reference solutions were prepared by diluting the stock solutions with water containing 1.5 ml concentrated nitric acid/litre. Calibration curve for each metal was prepared by
plotting the absorbance of standards versus their concentrations.

**Serum digestion and analysis for heavy metal**
With a micropipette, 1 ml of serum was measured into a tube to which 1 ml of nitric acid was added. The mixture was properly mixed and boiled at 100°C for 30 min and after which distilled water was added up to 10 ml mark. The concentration of heavy metals was measured using FS240AA Agilent Atomic Absorption Spectrophometer (21).

**Statistical analysis**
The results were expressed as the mean ± standard deviation (SD). Data were analyzed using SPSS and excel. The mean comparison was performed between group using the one way ANOVA at 95% confidence level.

**Results**

**Concentration of heavy metals in the kaolin samples.**
The analysis of the kaolin samples revealed the concentration of the heavy metals as follows: Lead--0.347 ± 0.12 mg/kg, Arsenic--0.354 ± 0.15 mg/kg, Cadmium--0.207 ± 0.11 mg/kg and Mercury--0.029 ± 0.02 mg/kg. The concentration of Lead (Pb), Arsenic (As) and Cadmium (Cd) were significantly (p < 0.05) higher than the maximum permissible concentration of the metals (Tables 1).

**Table 1:** Mean concentration of heavy metals in kaolin compared with maximum permissible concentration in food

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Mean conc. of heavy in kaolin (mg/kg)</th>
<th>Max permissible conc in food (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>0.347 ± 0.12</td>
<td>0.2</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.029 ± 0.02</td>
<td>0.3</td>
</tr>
<tr>
<td>Arsenic</td>
<td>0.354 ± 0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.207 ± 0.11</td>
<td>0.1</td>
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</tbody>
</table>

- P < 0.05

**Effect of kaolin consumption on serum heavy metals in 1st trimester pregnant women**
The heavy metals concentrations were elevated in almost all the groups. The serum concentrations of lead in Groups B and C were significantly (p < 0.05) elevated while the concentrations of arsenic, cadmium and mercury were not significantly (p > 0.05) different in all the groups when compared to the control group (Figure 1).

**Effect of kaolin consumption on serum heavy metals in 2nd trimester pregnant women**
The concentrations of heavy metals in kaolin consuming pregnant women are presented in figure 2. In the geophagic 2nd trimester pregnant women, serum levels of the heavy metals were elevated in almost all the groups. The concentrations of Lead in groups B and C were significantly (p < 0.05) increased when compared with the control (Figure 2). The concentrations of Arsenic and Cadmium were significantly (p < 0.05) raised in group C while the concentration of Mercury was not significantly (p > 0.05) different in all the groups.

**Figure 1:** The effect of kaolin consumption on serum heavy metals in 1st trimester pregnant women. *P* < 0.05. Pb-lead, As-arsenic, Cd- cadmium, Hg-mercury. Group A – 100 g of kaolin/week, Group B-150 g of kaolin/week and Group C- 200 g of kaolin/week
As men, anabdomths, microbes and heavy metals nd 23ly contaminated with lead but also with Hg). Acute te, 9Group C is bioavailable and Group B concentration in their urine even below μg/ml d exposure (28 Cd–
35ant women and their fetuses –, as well as on individual Hg–
rt of controlead, eavy metal st have been well thin the eproductive effects, such as
30). Maternal arsenic exposure in early pregnancy has been associated with low birth weight and children with arsenic concentration in their urine even below limit of 50 μg/ml suffered impaired cognitive function (31). Some studies have however, indicated that the toxicity of arsenic depends on the exposure dose, frequency and duration, the biological species, age, and gender, as well as on individual susceptibilities, genetic and nutritional factors (32). The. Arsenic is a protoplastic poison since it affects primarily the sulphhyl group of cells causing malfunctioning of cell respiration, cell enzymes and mitosis (33).

Cadmium is a highly toxic nonessential heavy metal that is well recognized for its adverse influence on the enzymatic systems of cells, oxidative stress and for inducing nutritional deficiency (34). Cadmium has the capability to bind with cystein, glutamate, histidine and aspartate ligands and can lead to the deficiency of iron (35). Cadmium and zinc have the same oxidation states and hence cadmium can replace zinc present in metallothionein, thereby inhibiting it from acting as a free radical scavenger within the cell. Increased concentration of cadmium has been found in the placenta of women who have given birth to children with low birth weight, neural damage and Down’s syndrome. Children who are exposed to large concentrations of cadmium in their environment often have learning disabilities (36).

Discussion
The result of this study revealed that the mean concentration of Lead, Arsenic and Cadmium in kaolin consumed by pregnant women in Anambra State Nigeria exceeded the maximum permissible concentration in food. This is in agreement with previous finding (9). An abnormally high level of lead and cadmium had also been reported in kaolin from other towns in Anambra State (23). Obviously, kaolin consumed by pregnant mothers in this study is contaminated by heavy metals, and this exposes both the mother and foetus to high risk of heavy metal toxicity with the attendant health consequences especially to the developing brain (24). Previous results had revealed that kaolin in the local markets were not only contaminated with lead but also with cadmium and arsenic (23, 25).

The significant increase in serum lead concentration in both 1st and 2nd trimester pregnancy of geophagic women is in tandem with the preclinical report of Bonglaisin, (26). Lead in kaolin is bioavailable and chronic kaolin consumers like pregnant mothers are therefore at higher risk of lead toxicity. The adverse effects of lead in children have been well documented (27). Reproductive effects, such as spontaneous abortions in women have been associated with high lead exposure (28). Acute exposure to lead induces brain damage, kidney damage, and gastrointestinal diseases, while chronic exposure may cause adverse effects on the blood, central nervous system, blood pressure, kidneys, and vitamin D metabolism (29, 30).

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Conclusion
Most of the kaolin sold in our local markets contains significant amount of lead, arsenic and cadmium, which are considered toxic especially to pregnant women. Kaolin consumption in pregnancy could be a risk factor for heavy metal toxicity and should be discouraged. The clinical outcome of kaolin consumption on pregnant women and their fetuses should be strictly monitored.

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