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Assessment of Haemoglobin Status and Transplacental Transport of Lead and Calcium During Geophagy

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Abstract

The consumption of Pb contaminated kaolin can be linked to fetal Pb exposure since Pb mimics calcium in its assimilation mechanism or active transfer to the foetus. Exposure to Pb equally increases susceptibility to iron deficiency. The study occurred postpartum to determine Pb and calcium in cord blood as well as the Hb levels of 54 pregnant women consuming kaolin. They included; 15 habitual consumers of kaolin, 15 previous consumers of kaolin and 24 non-consumers of kaolin. Statgraphic 5.0 was used for data analyses. Neonatal cord blood Pb values of the subjects starts from 0 µg/100 g in habitual consumers of kaolin, increasing to a mean value of 76.2 ± 59 µg/100 g for non-consumers of kaolin then to 178.6 ± 88.4 µg/100g of whole blood for previous consumers of kaolin with statistical significance between the groups (p=0.001). Kaolin-eating was associated with modest increase of calcium in cord blood of habitual consumers of kaolin correlating negatively to Pb (r=-0.99). Hemoglobin values increased from habitual consumers of kaolin (10.6 g/dl) through previous consumers of kaolin (12.3 g/dl) to non-consumers of kaolin (13.03 g/dl). This study reveals that Pb does not pass into cord blood during kaolin-eating but does so for previous consumers of kaolin. Local kaolin-eating leads to low Hb level in human.

Keywords: Kaolin; Lead (Pb); Calcium (Ca); Haemoglobin (Hb); Pregnant women

Introduction

Human placentas from pregnant women with high blood levels of Pb have been reported to be equally high in Pb levels by several authors [1-3]. Such increase blood Pb derived from Pb contaminated foods and sometimes largely from maternal bone stores due to long-lived stores of Pb in these issues has been proven to impair fetal and infant development [4] as placental barrier is permeable to free serum or plasma Pb [5-8]. The consumption of kaolin might bring about such Pb placental movement as it has been reported that kaolin is contaminated with Pb [9-10]. Also, Pb present in kaolin is bioavailable and passes into the fetuses of albino rats [11]. The effect this metal has on the human fetus sparks a need to fill the knowledge gap on the index of Pb in cord blood of kaolin consumers.

Individuals exposed to Pb are susceptible to iron deficiency [12], a condition that is common in the United States [13]. It is biologically plausible that iron deficiency could lead to higher Pb levels in human subjects exposed to Pb. It is also possible that iron deficiency modifies behavior, increasing pica, geophagy or hand-to-mouth behavior in children and thereby increasing ingestion to Pb in their environment [14].

Controlled animal studies consistently demonstrate higher Pb levels in iron-deficient animals than iron-replete ones suggesting higher Pb absorption in the former [15-16]. Similarly, it is known that Pb^{+2} can occupy vacant Fe^{+2} sites in the hermtopocitic system, thereby reducing Pb excretion. Clinical studies of chelation therapy suggest that iron deficient children may retain more Pb in their bodies [17-18].

Despite the similarity of results in animal studies, the findings in human studies are not consistent. Experimental studies of iron deficiency and Pb uptake in human are not consistent [18-19]. While several epidemiological studies in human subjects support a correlation between iron deficiency and higher blood Pb [20-21], others have not found any relationship between iron intake or low iron stores and blood Pb in human [22-23]. In the phase of this discrepancy it is not known to what extent the consumption of Pb contaminated kaolin by pregnant women in Cameroon may be affecting their Fe status.

Lead (Pb) mimics calcium in its assimilation mechanism [24] and is transferred in a pattern similar to that of calcium at the level of the placenta [25-27]. Calcium is highly required for the development of the fetus especially during the last trimester of pregnancy [28]. Hypocalcaemia provokes kaolin consumption [29] to meet calcium requirements [30] that may also lead to Pb intake [10]. Given this causal relationship, the question arises as to the possible association between blood calcium status of pregnant women and fetal cord blood levels. This is the question that needs to be answered by comparing the level of cord calcium to that of Pb since Pb has been observed not to pass into cord blood during moderate kaolin consumption in albino rats [11].

This investigation occurred postpartum to determine Pb and calcium in cord blood as well as the hemoglobin levels of females consuming kaolin; comparing findings to previous and non-consumers of kaolin.

Materials and Methods

This was a follow-up study after that on albino rats [11]. It was carried out with the approval and in accordance with ethical clearance of the Institutional Review Board (IRB) of the Cameroon Baptist Convention. Information on the sale and purchase of kaolin was obtained from kaolin merchants in Kumbo market.

The investigation started with a pilot study made of 64 respondents in the Bamenda area and the prevalence of geophagy amongst pregnant women found to be 82.5%. This prevalence was used to calculate the sample size. A formula of calculating sample size (at 95% confidence level) in epidemiological studies was used [31].

Pregnant women attending Antenatal Clinic (ANC) were recruited after informed consent in Banso Baptist Hospital, Kumbo and classified after informal discussion into 3 groups namely; habitual kaolin consumers (HKC), previous kaolin consumers (PKC) and non-kaolin consumers (NKC). Subject recruited were only women in their last trimester of pregnancy, initially on iron and folic acid tablets and prophylaxis against infectious diseases such as malaria and hookwarms etc.

On the whole a total of 54 pregnant women gave their consent within the study period approved by the IRB. They included 15 HKC, 15 PKC and 24 NKC. Information that included: consumption, reasons for consumption, social class, age range, physiological effects of kaolin consumption, knowledge about the ban passed on kaolin consumption, etc. was collected by questionnaire.

Within two to four weeks before delivery 54 venous blood samples were collected from the subjects during ANC in ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes and analyzed for Hb level. At delivery whole blood was collected in EDTA tubes from fetal cords of their newborn.

A total of 54 samples of cord blood were collected from 54 women postpartum; 24 (44.4%) NKC, 15 (27.8%) HKC and 15 (27.8%) PKC (Table 1). Previous consumers (PKC) were those that had stopped eating kaolin either few months before pregnancy or after ANC education on the dangers of consuming this substance on health and the fetus. As regards consumers (PKC and HKC), 5 (16.7%) ate kaolin during pregnancy at the rate of two times per day, 20% ate this clay type at the rate of one time per day (before and during pregnancy) while 10% consumed it greater than two times per day. The rest (43.3%) were either casual consumers or those who couldn't remember their consumption rate. Of all who consumed, 40% did so for taste, 26.7% through influence by friends, 10% to alleviate nausea, 20% for therapeutic and 3.3% for no reason at all. Professionally the PKC and HKC can be categorized as follows: farmers 11 women (36.7%), working class 08 women (26.7%), trading 04 women (13.3%), students 05 women (16.7%) and housewives 02 women (6.7%). Age range in the study was 15-40 years with the age category of 21-25 years dominating (31.1%) for all the women in the study.

The consumption of kaolin was not without some immediate side effects. Physiological effects following the consumption of kaolin was found to range from feeling of heart burn and nausea (13.3%) through general discomfort (20%), and sickness (20%) to satisfaction (23.3%).

Collected cord blood samples were digested with concentrated nitric acid (HNO_3) for Pb and calcium [32]. All reagents used in the analysis were of analytical grade. Analyses were carried out using Perkin 311 model Atomic Absorption Spectrophotometer, as described by Burtis and Ashwood [33]. Air-acetylene gas was used as fuel, and wavelength used for cationic estimation of Pb was 283.3 nm while that for calcium was 422.7 nm. Concentrations of Pb (µg/g) and calcium (mg/L) of the cord blood samples were sorted on excel sheets using standard plot values and their absorbance by linear regression equation.

Entry investigated	Number	Outcome	Percentage
Pregnant women	24	NKC	44.4
	15	PKC	27.8
	15	HKC	27.8
Reason for consumption	12	Taste	40
	8	Influence of friends	26.7
	3	Nausea	10
	6	Therapeutic	20
	1	No reason	3.3
Consumption rate	6	01 time per day	20
	5	02 times per day	16.7
	3	>02 times per day	10
	13	Casual (less often)	43.3
Health problems encountered	4	Heart burn and nausea	13.3
Physiological effects	6	Release from discomfort	20
	6	Feeling bad	20
	7	Feeling good	23.3
	11	No effect	36.7
Ban awareness	41	Aware of the ban	75.9
	13	Not aware of the ban	24.1
Reaction after the ban	11	Stopped eating kaolin	36.7
	19	Ignored the ban	63.3
Profession of women involved	11	Farmer	36.7
	8	Working class	26.7
	4	Trading	13.3
	5	Students	16.7
	2	Housewife	6.7

Table 1: A descriptive analysis of the questionnaire.

Haemoglobin (Hb) levels of the subjects were determined by HemoCue [34] blood Hemoglobin system. Each venous blood sample was analyzed in quadruple and a mean value obtained.

Statistical analysis

The data obtained were subjected to a one-way Analysis of Variance (ANOVA) according to the procedure of Steel and Torrie [35] using

Statgraphic 5.0. Significantly different means were separated using the methods of Duncan [36]. The values obtained were presented as Least Significance Differences (LSD) of means at ($p<0.05$) compared to those which did not differ significantly ($p>0.05$) from the value of Duncan.

Results and Discussions

Questionnaire on pregnant women

Although there exist a ban by the Ministry of Public Health on the consumption of kaolin, it was observed that about 3 out of every four pregnant women interviewed were aware of its ban while 1 out every 3 who knew of the ban had stopped consuming kaolin. Consumption quantities were 25 and 50 FRS CFA corresponding approximately to 30 g and 60 g of kaolin consumed respectively.

Studies have confirmed that geophagy during pregnancy is linked to nausea or vomiting associated with morning sickness [37], taste [37-38] influence from others [39-40] and therapeutic where clay or soil is considered like a medicament [39]. Rate of consumption that varied during pregnancy from daily to casual, with occasional or casual consumption dominating is consistent with [39]. Geophagy has been reported amongst different professions [39], and it is observed not to be linked to any specific profession like in this study. In addition to feeling good or bad and release from discomfort, another physiological effect that has been reported is hunger [37].

Cord blood Pb content

As seen in Table 2, the quantity of lead (Pb) in the neonatal cord blood of women involved in the study starts from zero in HKC, increasing to a mean value of 76.2 ± 59 µg/100 g of whole blood for NKC then to 178.6 ± 88.4 µg/100 g of whole blood for PKC with statistical significance between the groups ($p=0.001$).

Pregnant women	Data expressed (% of Pb values above zero)	Pb (µg/g of cord whole mean blood)
NKC	25	76.2 ± 59.0
PKC	26.7	178.6 ± 88.4
HKC	0	00.0
Values in the same column having the same superscripts are not significantly different ($p>0.05$)		

Table 2: Evaluation of Pb content of cord whole blood of babies born to women of different history of kaolin consumption.

The standard elevated blood lead level (BLL) for adults set by the Center for Disease Control (CDC) is 25 micrograms per deciliter (25 µg/dl) i.e. 25 µg/100 g of whole blood. The level for a child is much lower; currently it is 10 micrograms per deciliter (10 µg/dl or 10 µg/100 g) of blood. This latter value for whole blood was adopted by CDC in 1991 as an action level for children, an advisory level for environmental and educational intervention [41]. Also, World Health Organization (WHO) has sets standards of blood lead level not to exceed 100 µg/L i.e. 10 µg/100 g of whole blood [42]. The National Institute for Occupational Safety and Health (NIOSH) in the United States precised that a blood lead value above 40 µg/100 g is indicative of excess exposure and one above 60 µg/100 g requires removal from exposure [43]. It therefore holds that the cord blood values of 178.6 ± 88.4 µg/100 g and 76.2 ± 59 µg/100 g of whole blood for 26.7% and 25% of PKC and NKC respectively are above the acceptable limit of 10 µg/100 g for whole blood set by WHO, indicating excessive exposure that requires removal from exposure as stated above. Standard deviations (Table 2) indicate that there are variations in Pb values amongst PKC and NKC.

Calcium concentration in whole blood obtained of babies born to women with different history of kaolin consumption

Averages and ranges on Table 3 show that kaolin consumption was associated with modest increase of calcium in cord whole blood of HKC.

Kaolin from Nigeria constitutes about 83.6% [10] of local kaolin available in the Cameroon market. Studies by Talabi et al., [44] had reported that kaolin from Achala-Agu (Nigeria) contains high levels of calcium, 6.23 ± 0.43 g/100 g in calcium oxides found [44]. On the basis of these results, the consumption of 100 g of this kaolin provides about 4450 mg of calcium.

Pregnant women	Count	Ca (mg/l of Cord whole blood)	
		Average	Range
NKC	22	11.54	0.70 – 26.0
PKC	15	10.29	0.70 – 23.4
HKC	15	18.7	10.3 – 27.9

Calcium estimates were based on consumption quantities that were 25 and 50 FRS CFA for pregnant women in BBH (corresponding approximately to 30g and 60g of kaolin consumed respectively). Calcium intake for 30 g corresponds to=(30 × 4450)/100=1335 mg. Similarly, calcium intake for 60 g corresponds to (60 × 4450)/100=2670 mg (Figure 1).

Balengou clay discovered to be made up principally of halloysite (70%) also contains CaO oxide at 0.06 g/100 g or 60 mg/100 g of halloysite [45]. Calcium intake for 30 g corresponds to 12.9 mg and calcium intake for 60 g corresponds to 25.8 mg (Figure 1). Kaolin from Nigeria and Balengou were the most preponderant in the local market.

The daily requirement for calcium is set at 1,300 mg/day for pregnant women [46] indicating that local kaolin from Nigeria contains calcium above this recommended level and consuming only kaolin for 25 FRS CFA will meet the daily requirement for calcium. Clay from Balengou will provide very little calcium, far below the daily

requirement in calcium, though its effect on the population will not be preponderant as it constitutes only 11.5%.

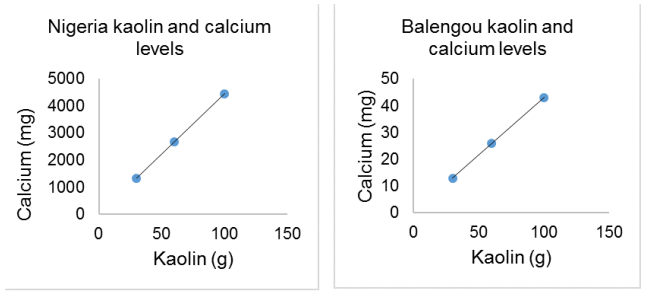

Figure 1: Calcium content of kaolin in the local market (Achala-Agu, Balengou).

A greater percentage of calcium in the diet is absorbed during the third trimester of pregnancy [47], indicating increased need of calcium for the foetus at the prenatal period. Therefore dietary/kaolin sources of calcium are absorbed more during this period, confirming the high level calcium observed at the level of the cord especially for current consumers of kaolin. Authors have observed that, 99% of the flow of calcium is maternal-to-fetal [48], and this active, one-way process is under way by the third (last) trimester, when the majority of calcium is transferred, with the fetus accumulating about 250–350 mg/day [49-50].

Correlation of Pb and calcium concentrations in cord whole blood

Figure 2 shows that mean concentrations of Pb and calcium were inversely correlated, as indicated by the coefficient of linear correlation (r=-0.99). The passage of Pb into and through the placenta is slightly inversely related to trans-placental transport of calcium at that level. Similar interaction of Pb and Ca from dietary sources has been reported during absorption in which high level of Ca was observed to precipitate Pb in the intestinal lumen of rats [51]. It is known that poor dietary calcium intake is associated with lead accumulation in blood and organs [52], including bone tissue.

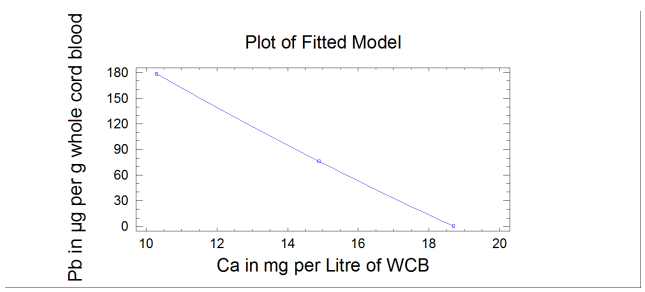

Figure 2: Correlation of Pb and Ca in cord whole blood.

The findings of this study reveal that many women continue to consume local kaolin after prenatal education they receive to avoid kaolin consumption especially during pregnancy. Surprisingly, the index of lead (Pb) in the cord blood from children of HKC and PKC pregnant women compared to that of NKC was statistically insignificant as observed in rat [11]. Lead has been observed to pass into rat blood stream even at low quantities of kaolin consumption [11]. This is evidence of Pb bioavailability, though correlation of animal findings that predict human response to chemical substances is still a contentious issue. Thus, research result like the one on rats would only be hypothetical about the likely human response to these heavy metals. But this hypothesis could not be verified due to ethical consideration because kaolin consumption has been banned within the confines of this research.

However, cord blood Pb finding of 0 µg/g in rat that coincides with that in human, pushes one to imagine a similar Pb bioavailability scenario in human during the consumption of Pb contaminated kaolin. If this is the case, then a metabolite or mineral is controlling the release of bone lead as well as the passage of blood or plasma lead (Pb) into the placenta during kaolin consumption. Is calcium the mineral substance responsible? Kaolin is rich in calcium [53-54], especially that from Nigeria [44]. This fact is probable because prevention of cramp (tetany) due to hypocalcaemia by kaolin or clay has been reported by earlier authors [29] and whole cord blood of current consumers of kaolin was observed to contain more calcium (18.7 mg/L), when compared to non-consumers (11.54 mg/L) and previous consumers (10.29 mg/L) of kaolin in a statistical significant manner. Though this was not a control study as women ate kaolin in different quantities and sometimes at different time intervals, it seems reasonable to state that lead (Pb) affinity for bone tissue may be higher than for the placenta or that all the lead (Pb) getting into blood from kaolin source may be going elsewhere except the placenta and may not be released because of adequate calcium. This is obvious because there has been evidence of Pb being released from Pb stores (such as bone) during breastfeeding in albino rats [11]. Therefore the release of bone Pb during pregnancy (and probably old age) can be suppressed by an increased intake of calcium. From this viewpoint, continuous consumption of kaolin is a solution, except for the fact that Pb passes into breast milk during breastfeeding [11]. However, since Pb in local kaolin is observed to be bioavailable, HKC still run the potential risk of spontaneous abortion and increased blood pressure that affect a community under Pb exposure as reported by [54], together with the classical signs of lead poisoning, even if Pb doesn't affect their fetuses. Its effects on women include infertility, miscarriage, premature membrane rupture and premature delivery [55]. McMichael [55] revealed that lead may be toxic at levels previously thought to have no effect. The U.S. Public Health Services stated that there is no safe level for lead and as a practical measure recommended reduction of blood lead levels to less than 10 µg/100 g in women of childbearing age. In fact, lead can cause serious problems on the kidneys, blood cells, nerve cells, gut, bones and hormonal imbalance [56-57].

In addition, a value of 178.6 ± 88.4 µg/100 g of Pb in whole blood for 26.7% PKC suggests that fetuses of kaolin consumers will become vulnerable immediately their mothers stop the consumption of this clay - perhaps for other reasons (e.g. its effects on hemoglobin as presented below), as lead (Pb) stores in the bones would be released into the blood stream during pregnancy as well as in subsequent pregnancies or old age.

A value of 76.2 ± 59 µg/100 g of Pb in whole blood of NKC (26.7%) suggests that they may have taken in this heavy metal from its other contaminants in our ecosystem or food chains. Similar results were found in women presumed to be healthy by researchers [58]. The fetuses of this category are also exposed to Pb intoxication, with manifestations such as low birth weight, fetal hypotrophy and

malformation [59]. Though there are other manifestations that are not seen until several years after birth such as retarded mental development and muscular and behavioral disorders [54].

Hemoglobin level of women involved in the study

As can be observed on Table 4, the hemoglobin levels of the pregnant women in the study increased from kaolin consumers (10.6 g/dl of blood) through previous consumers (12.3 g/dl of blood) to non-consumers (13.03 g/dl of blood). Hemoglobin cutoff used to define anemia in pregnant women living at sea level is 11 g/dl [59]. According to this cutoff, pregnant women who are either non-consumer or previous consumers of kaolin (with hemoglobin values of 13.03 g/dl and 12.25 g/dl respectively) are not anemic while those who are consumers of kaolin (with hemoglobin value of 10.59 g/dl) are considered to suffer from anemia. Ranges (Table 4) also indicate high anemia amongst kaolin consumers compared to previous and non-consumers of kaolin.

Pregnant women	Count	Hemoglobin content (g/dl)	
		Average	Range
NKC	24	13.03	10.80 – 16.6
PKC	15	12.25	11.30 – 13.3
HKC	15	12.03	6.90 – 12.2

Table 4: Evaluation of the Haemoglobin content of pregnant women.

High prevalence of anemia in pregnant women, especially migrant women practicing geophagia in Johannesburg has been found [39], confirming that kaolin consumption can negatively affects Hb level of the body. These findings also coincide with those of the previous authors [60- 62] that revealed a positive correlation between kaolin consumption and anemia. Where severe anemia is common, there is also an increase in the number of maternal mortality and obstetrical complications indicating that low Hb level during pregnancy results to undesired outcomes. The hemoglobin characteristics though without hematocrit, erythrocyte counts and serum ferritin levels determination are suggestive of iron deficiency and/or intoxication from Pb because the ANC women involved were on follow up against infectious diseases that would bring about blood loss.

The targets of Pb and kaolin effects on iron are the bone marrow/red blood cells and intestines respectively [63]. Since exposure to Pb is toxic to the bone marrow, low Hb level will be due to low red blood cells from the bone marrow and stem cells, a common scenario in microcytic anemia. Kaolin is also known to form complexes with iron leading to non-absorbable iron compounds at the level of the intestines [61-62]. In the latter case, low Hb level maybe be due to the hindering of the uptake of dietary iron, a common situation in iron deficiency. In the present study these two phenomena are obvious as pernicious or megaloblastic anemia can be ruled out because subjects were on routine intake of folate tablets and vitamin B12 as recommended during antenatal education.

It is also established that Pb has very high affinity for red blood cells (erythrocytes) and Pb toxicity is associated with saturnism, a disease characterized by anemia and peripheral neuropathy [64-65]; it has been shown that lead inhibits the enzymes Amino Levulinic Acid Dehydratase (ALAD) and ferrochelatase of the heme synthetic pathway thus preventing conversion of ALA to porphobilinogen and inhibits incorporation of iron into the protoporphyrin ring respectively. This would result to reduced heme synthesis or anaemia, with outcome being an elevated level of the Amino Levulinic Acid (ALA) precursor, which is a weak γ-Amino Butyric Acid (GABA) agonist that decreases GABA release by presynaptic inhibition [66] with nervous disease as consequence. The synergic effect of contaminated kaolin and its Pb content is thus rendering pregnant women that consume kaolin pruned to this condition because of their eating disorders, compromising the efforts of the Ministry of Public Health that has advocated and ensured that iron and folate tablets are taken during pregnancy to curb down anemia.

Conclusion

The results seem to be accord with the following conclusions:

Lead (Pb) does not pass into cord blood (in pregnancy) during the consumption of low kaolin quantity.

Cord blood calcium levels are highest amongst the current consumers of kaolin and trans-placental Pb transport negatively correlates with calcium profile of cord blood.

Bone lead (Pb) of the previous kaolin consumers is released during pregnancy into the blood stream with the passage into cord blood confirmed, rendering the women and their foetuses vulnerable to lead toxicity.

The study also revealed high values of lead in the cord blood of some non-kaolin consumers strongly suggesting that there may be other sources of lead (Pb) contamination in our food systems or environment (ecosystem). And the consumption of Pb contaminated kaolin brings about low Hb in the body in adequate dietary iron.

However, more studies are needed to confirm the findings given that Groups are very small and heterogeneous in consumption of Kaolin and characteristics

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