Effect of Lead Exposure on Anaemia Among Children Attending the University of Ruhuna Teaching Hospital in Karapitiya, Sri Lanka: A Pilot Study

by

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William Pan

Thesis submitted in partial fulfillment of
the requirements for the degree of Master of Science in the
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ABSTRACT

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Abstract

Background: There is limited recent data on anaemia in Sri Lanka, however previous estimates put prevalence at 25-35% in children under 5, approximately 16.3% among 5-9 year olds and 9.9-13.9% among 10-15 year olds. Iron deficiency is often cited as the most common cause of anaemia, however in Sri Lanka most anaemia cases are not iron deficiency anaemia related and alternative aetiology related to other causal factors such as heavy metals should be explored. Exposure to heavy metals has been linked with anaemia in other settings and it is plausible that it may contribute to anaemia in Sri Lanka. *Aims & Methods:* This study is a comparative cross-sectional study setting out to explore the relationship between exposure to lead and anaemia as well as the influence of other factors. Concentration of lead in hair samples and information regarding basic demographic characteristics was collected from children ages 2 to 14 with and without anaemia. Patients were randomly convenience sampled from the outpatient and inpatient paediatric clinics in Karapitiya teaching hospital. *Results:* The only predictor variables independently associated with having anaemia and lower haemoglobin were male gender and under 5 age. A logistic regression model with anaemia as the outcome found that females were significantly less likely to be anaemic (OR=0.122, p=0.0045) and overweight children were also significantly less likely to be anaemic (OR=0.777, p=0.032). A multivariable regression model assessing contributing factors including age, gender, ethnicity, body mass index (BMI) and stunting could only explain 18.44% of change in haemoglobin levels. Only female gender and age over 5 was significantly associated with higher haemoglobin levels (Coeff=0.932, p=0.015; Coeff=0.787, p=0.01). Tamil ethnicity in this model was associated with significantly lower haemoglobin levels (Coeff= -1.613, p=0.020). *Conclusion:* Haemoglobin levels, were expected to be negatively correlated with hair lead levels. However, due to the unforeseeable and persistent breakdown of the laboratory equipment the lead content of the collected and processed hair samples could not be analyzed by the thesis deadline. In order to enable and complete similar research in the future, strengthening of local laboratory capacity is suggested.

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1. Introduction

Anaemia is an important global health problem affecting approximately 32.9% of global population, corresponding to more than 2.2 billion people with the highest number of the affected living in South East Asia (WHO 2008b, Kassebaum et al. 2014). This burden of disease attributable to anaemia, accounted for 68.4 million Years Lived with Disability (YLD) in 2010. More specifically South East Asia accounted for 37.5% of global YLD due to anaemia, indicating that this is an important issue in this region (Kassebaum et al. 2014). Up to 60% of women, 36% of men and 66% of children are anaemic in South East Asia. Furthermore, anaemia contributes to approximately 324,000 deaths and 12,500,000 Disability Adjusted Life Years (DALYs) per year in this region (Kotecha 2011). Anaemia is often overlooked because there are no obvious symptoms. However, not only does anaemia contribute to morbidity and premature mortality but it may also result in a loss of productivity and therefore massive economic loss (WHO 2008b). Some sources have suggested that the global prevalence of anaemia has decreased, however a global estimate on prevalence made in 1985 suggested that approximately 30% of global population suffered from anaemia, which is similar to current estimates made by the WHO and indicates that the prevalence of anaemia has hovered at around the same number for the past few decades (Kassebaum et al. 2014, WHO 2008b). Economic loss due to anaemia is often quantified as loss of work productivity and adverse cognitive effects in children. Overall, the productivity loss accumulated in three South Asian countries (Bangladesh, India, and Pakistan), is estimated to be as high as $4.2 billion annually (Balarajan et al. 2011, Horton and Ross 2003).

"Anaemia is the condition where red blood cells, or their capacity to carry oxygen is impaired, to the point that physiological needs of the human body are no longer adequately met" (Amarasinghe et al. 2017). Normal haemoglobin levels vary by age, sex, ethnicity and health status (Mettananda and de Silva 2017). Anaemia is broadly classified by two causes: ineffective erythropoiesis which is decreased red blood cell production or haemolysis which is the increased loss of red blood cells or both (Balarajan et al. 2011). Anaemia is most common in young children and has varying aetiologies and complex, difficult to control characteristics which has led to many studies seeking to identify reasons for high prevalence and associated factors among such age groups (Zuffo et al. 2016). While iron deficiency is acknowledged as a significant cause, other attributable factors include heavy blood loss due to menstruation in females, parasitic infections, micronutrient deficiencies, environmental exposures to heavy metals and acute and chronic conditions such as malaria, cancer, tuberculosis and HIV (WHO 2008b, Hegazy et al. 2010).

Anaemia is also often associated with decreases in trace elements such as iron, zinc and copper (Hegazy et al. 2010). An association between anaemia and exposure to heavy metals has also been described in previous studies (Weinhouse et al. 2017, Jain et al. 2005, Olivero-Verbel et al. 2007). The US EPA suggests a blood lead threshold level of 30-40μg/dl is associated with increased chances of anaemia in children (EPA 2004). Lead is known to cause anaemia through both erythropoiesis and haemolysis by interfering with enzymes involved in the formation of erythrocytes as well as shortening the lifespan of erythrocytes (Jacob et al. 2000, Balarajan et al. 2011).

There is limited recent survey data regarding anaemia from Sri Lanka, with the most recent data coming from the National Nutrition and Micronutrient Survey in 2012, which covers only children under 5. This survey estimated that 15.1% of children were anaemic ranging from 4.9% to 26.9% among various districts (Jayatissa, Gunathilaka and Fernando 2012). A more comprehensive review of nutritional status in Sri Lanka compiled in 2011, indicated that prevalence of anaemia ranged from 25-35% in children under 5, approximately 16.3% among 5-9 year olds and 9.9-13.9% among 10-15 year olds. Anaemia is often linked to nutritional factors and micronutrient deficiencies. Approximately half of the population in Sri Lanka does not consume the minimum level of calories per day and additionally, dietary diversity is low (Rajapaksa, Arambepola and Gunawardena 2011). To date, there are no studies linking lead exposure and anaemia in Sri Lanka, although such studies have been conducted in nearby India which found that elevated blood lead levels of >10μg/dl were associated with risk of moderate and severe anaemia (Jain et al. 2005, Ahamed et al. 2007).

Addressing the determinants of anaemia is challenging due to the complexity of interactions between causal factors. Therefore it is crucial to reduce knowledge gaps through research to improve policy and interventions and reduce the burden of anaemia (Balarajan et al. 2011).

1.1 Justification

Anaemia is a significant public health issue, contributing to the global burden of disease and impacting social and economic development in both developed and developing countries. The highest prevalence is found in pre-school aged children and the lowest in men. While iron deficiency is acknowledged to be a significant contributor to anaemia, it is estimated that up to 50% of anaemia cases are not caused by iron deficiency and alternative aetiological factors must be considered (WHO 2008b). There is a need to explore alternative causes of anaemia and to distinguish between anaemia caused by iron deficiency, and anaemia of alternative aetiology in order to develop appropriate strategies to control the issue (Weinhouse et al. 2017).

According to the WHO estimates in 2008, anaemia in Sri Lanka has a “moderate” public health significance (WHO 2008b). The National Micronutrient Survey of 2012 in Sri Lanka found an overall national prevalence of 15.1% in Sri Lankan children with the highest prevalence in the Kilinochchi district at 26.9% and the lowest prevalence in the Kegalle district at 4.9% (Mettananda and de Silva 2017, Jayatissa et al. 2012). The same survey found that the national prevalence of anaemia strictly due to iron deficiency was approximately only 7.3% in children ages 6-59 months, meaning that very few anaemia cases were in fact due to iron deficiency (Jayatissa et al. 2015). A study of Sri Lankan children by Amarasinghe et al found that only two anaemic children out of 417 had depleted iron stores. This study along with other previous studies, suggests that iron deficiency is not necessarily the only cause (Amarasinghe et al. 2017, Mettananda and de Silva 2017).

Children are more vulnerable to lead exposure due to behavioural patterns and the level of absorption of the lead they are exposed to (Shah et al. 2010). Once lead is ingested, it is absorbed from the gastrointestinal tract at a higher percent of ingested dose in children (up to 40%) than adults (5-10%) (Kaji and Nishi 2006). In Sri Lanka, lead levels have not been extensively studied. A literature review yielded one paper from 2004 comparing blood lead levels of children 1-15 years old before and after the introduction of unleaded petrol (Senanayake, Rodrigo and Malkanthi 2004). This study will provide more recent data on the situation.

To date, three main public health interventions are in place to combat anaemia to date: multiple micronutrient supplementation, intermittent iron/folate supplementation and anti-helminthic treatment (Mettananda and de Silva 2017). However, infection by parasites is quite uncommon in Sri Lanka since recent improvements in sanitation and hygiene. Also, a previous study found the prevalence of iron deficiency anaemia is only 7.3%, and universal supplementation of iron is only recommended in settings with prevalence of over 40%. Unnecessary iron supplementation leads to adverse health affects such as increased risk of infections (Mettananda and de Silva 2017). This indicates, current policies and interventions do not address the true causes of anaemia and may not be the most effective use of resources. Also, should lead contribute to and exacerbate anaemia, there are currently no policies and/or interventions in place.

1.2 Evidence of Toxicity of Lead

Lead is a toxic heavy metal naturally present in the environment and emitted by various sources including mining and smelting, leaded petrol (in settings where this source of fuel is still used), leaded pipes, recycling of car batteries, toys and trinkets, glazed ceramics and old lead paints (WHO 2007). Other activities which release lead into the environment include using lead-containing wastewater for irrigation, inappropriate disposal of contaminated sludge and industrial activities (Wang et al. 2017). The WHO has defined the maximum acceptable concentration of lead in drinking water as 0.01 mg/l, but thresholds for other routes of exposure are more difficult to clearly define and regulate. Heavy metals accumulate in soil and other biological organisms over time. Crops raised in contaminated soils provide a pathway of exposure for heavy metals through diet and most populations are exposed to lead through the consumption of contaminated plant and animal products (Wang et al. 2017). Alternative exposure pathways for humans besides ingestion, includes inhalation and skin absorption. For small children, ingestion is the main source of exposure due to behavioural patterns (Kwok 2007).

Lead has no known function in human physiology or biology, and even very small concentrations are harmful due to bioaccumulation. Lead poisoning has several effects such as inhibition of the synthesis of haemoglobin, damage to the central nervous system and peripheral nervous system, and dysfunction of joints, kidneys and reproductive systems (WHO 2010). A common clinical manifestation of lead exposure is anaemia. Some studies have found that as serum lead levels increase, haemoglobin concentrations decrease (Turgut et al. 2007). The mechanism of anaemia by chronic lead exposure is primarily the impairment of haeme synthesis which diminishes the number of red blood cells in circulation, however a decrease in the survival and destruction of red blood cells may also occur (WHO 2010, Hsieh et al. 2017).

Chronic low-level exposure to lead has adverse neurological effects and children are especially vulnerable as they are still developing (WHO 2010, Kwok 2007). Key biological mechanisms of lead induced cognitive impairment include "disruption of key molecules during neuronal migration and differentiation, interference with synapse formation and premature differentiation of glial cells." These alterations of the nervous system occur especially during the prenatal period and through childhood and have long-term effects (Mason, Harp and Han 2014). There is currently no known threshold of lead exposure where detrimental developmental effects don't occur (Kwok 2007, WHO 2010). Epidemiological studies have found lead induced IQ decrements correlated to even low blood lead levels. In fact, loss of IQ was even higher at blood lead levels lower than 10μg/dl (WHO 2007, WHO 2010). A study analyzing NHANES III data in the US to test cognitive functioning in children ages 6-16 years with low blood lead levels found an inverse relationship between blood lead levels and cognitive function assessed by tests of arithmetic skills, reading skills, nonverbal reasoning and short term memory (Lanphear et al. 2000).

Close relationships between child development and lead levels exist not only intellectually but also physically. Previous studies have reported an association between stunting and chronic lead exposure. Stunting is considered an indicator of chronic malnutrition because it is an accumulation and reflection of long-term nutritional deficiency (Anticona and San Sebastian 2014, WHO 2008a). While the mechanism for the negative impacts of lead on child growth is unclear, it has been hypothesised that lead affects bone growth by interfering in the way vitamin D is metabolised and thus the way calcium is distributed in the body (Anticona and San Sebastian 2014). Adverse developmental effects may also have some economic repercussions, for example in the US, childhood lead poisoning may cost up to 43 billion USD per year. In addition, children suffering from the adverse developmental effects of chronic lead exposure may go on to lead less productive lives which is economically detrimental (WHO 2010).

In Sri Lanka, elevated lead levels have been found in soil samples whereby contamination of crops occurs. Local fish have also been found to have higher lead levels (Kananka and Gunaratne 2014, Premarathna, Indraratne and Hettiarachchi 2010). Sri Lanka has had a history of struggling with health issues linked with environmental factors such as Chronic Kidney Disease of uncertain aetiology (CKDu) (Diyabalanage et al. 2016). Heavy metals have been identified to act in synergism with other factors such as agrochemicals as a proposed disease causing mechanism (Rajapakse, Shivanthan and

Selvarajah 2016). Just like CKDu, anaemia has multiple causes and exposure to heavy metals is only one aetiological factor acting independently or in unison with others such as iron deficiency, infections and other micronutrient deficiencies.

1.3 Justification for Hair Analysis to Measure Lead Exposure

The presence of heavy metals in human hair can be used as an indicator of exposure to environmental pollution such as lead. Studies have found that the concentration of heavy metals found in hair are related to age, gender, ethnicity, eating habits and environmental factors (Wang et al. 2017). Several authors have reported that the analysis of hair is a reliable and good marker of heavy metal exposure (Wang et al. 2009, Llorente Ballesteros, Navarro Serrano and Izquierdo Álvarez 2017, Zhou et al. 2016). Since lead is a time varying exposure, analyzing hair samples has the advantage that it produces a record of a longer time period because hair stores and retains trace elements over longer periods of time while blood may only reflect a momentary fluctuation (Michalak, Wołowiec and Chojnacka 2014). Since relationships have been observed between the concentrations of pollutants in hair and other biomarkers such as blood, hair should be reflective of internal dose (Appenzeller and Tsatsakis 2012). In addition, the use of hair samples has the added advantages of low cost, painless sampling, easy storage and transport of samples and quick alternative to blood lead analysis (Onuwa, Ishaq and Sha'Ato 2012). In the context of this study, hair analysis was the most feasible and practical option.

Despite the above mentioned advantages of using hair samples in this study, there are several challenges and limitations. Estimating the exact dose presents a difficulty since it is not known at what rate substances within the body distribute into hair. Additionally, hair analysis does not differentiate between endogenous and exogenous exposure and it is not possible to determine its source of exposure (ATSDR 2003). Since this study is exploratory in nature and does not aim to determine the source or pathway of exposure, these limitations are somewhat negligible. Hair samples are not as frequently analyzed as blood lead levels, therefore there is a lack of standard guidelines. There is also no known baseline concentration of lead levels in hair of the general population in Sri Lanka, thus there is no way to conclude if lead levels observed are above or below average (ATSDR 2003, Zhou et al. 2016). Although this is a limitation on one hand, this study generates baseline information on hair lead levels in Sri Lanka's children and provides reference values.

1.4 Study Aims and Objectives

The main objective of this study was to investigate whether exposure to lead is associated with anaemia in children in Sri Lanka. To achieve this aim, hair samples were collected and analyzed for lead, and differences in distribution of lead and haemoglobin levels among children was assessed by some basic demographic characteristics. The study only included the following predictors: age, gender, ethnicity and height and weight data from which body mass index (BMI) and stunting is obtained. Other predictors could include socioeconomic status, education of mother and father as well as dietary diversity, however such data was not collected for the purpose of this study. It is hypothesized that there is an inverse relationship between lead levels and haemoglobin. Since there is a gap in data in this region regarding lead exposure in children, this study aims to fill this research gap.

A sub-aim of this study is to assess the impact of other predictors of haemoglobin levels among children in this setting including anthropometric measurements, gender, age and ethnicity.

2. Methods

2.1 Study Design and Setting

The study location was Karapitiya in the Southern Province of Sri Lanka. In terms of design, this study was a comparative cross-sectional pilot study. The recruitment period was between May-October 2018 and participants were recruited as either cases or controls according to haemoglobin levels determined at the time of recruitment. Biological samples (hair) were collected from each participant and a basic background questionnaire was filled out by the primary investigator or research assistant based on available medical records and information from parents.

2.2 Participant Enrolment

Since there is limited current information about the distribution of lead concentration in hair samples of children in Sri Lanka, a sample size calculation was not possible and target sample size was set at 100 just to establish a baseline. 50 children with anaemia were recruited as cases and 50 children without anaemia were recruited as controls according to WHO anaemia definitions. Children were recruited from the Karapitiya teaching hospital, a tertiary referral hospital, from the daily paediatric out-patient clinics as well as paediatric in-patient wards by using convenience sampling. Local medical records were used to identify children with and without anaemia by interpreting the most recent haemoglobin report using WHO standards. Children under 5 years with haemoglobin concentrations ≤ 11.0 g/dl were considered anaemic and children over 5 years with haemoglobin concentrations ≤11.4 g/dl were considered anaemic. Recruitment occurred in-person, as patients were coming in for a check-up or at the hospital bedside in the wards. Participating children and parents were fully informed of the purpose of this research and consent was obtained prior to the collection of information and samples. Medical records contained information regarding haemoglobin levels, diagnoses, medical history and anthropometric measurements. Children ages 2 to 14 years were included in the study. Since ingestion is considered a primary exposure pathway to lead in most populations (Hegazy et al. 2010), children of ages when feeding habits were suspected to be similar were selected. Children were excluded from the study if they had any known parasitic and/or acute infections or haemoglobinopathies which may lead to anaemia. Matching cases and controls was not possible with the time and resource constraints.

2.3 Sample Collection

Several strands of hair were cut from the occipital region near the scalp, the amount depending on the length of hair, using a pair of stainless steel scissors. Samples were stored in labeled paper envelopes until further processing. A code was assigned to each sample and no identifiable information was collected from participants. Hair samples were weighed at a later point in the lab during the processing to ensure that similar amounts of hair were processed. During the sampling process, parent(s) of the child were asked to provide basic information to fill out the remaining sections of the questionnaire for which no information was available in the medical records including ethnicity and area of residence. Data collected onsite at the hospital was recorded on paper and later transferred to the Duke University Redcap online data collection database.

2.4 Sample Processing

Hair samples were washed according to the process recommended by the International Atomic Energy Agency. Each hair sample was washed in a Pyrex beaker for a total of five 10 minute intervals involving mechanical shaking of the samples with 25ml portions successively of acetone, deionized water, deionized water, deionized water and acetone. The liquid was decanted off the sample after every 10 minute washing interval. After washing, each hair sample was dried overnight at room temperature. To digest the samples, wet acid digestion with nitric acid and hydrogen peroxide was used. According to literature, this non-automated method is superior in terms of accuracy, precision, recovery and method detection limit (Ishak et al. 2015). Samples were then transferred to acid resistant plastic bottles and transported to the Faculty of Fisheries and Marine Sciences and Technologies in Matara for lead analysis. The International Atomic Energy Agency (IAEA) accepts the use of atomic absorption spectrometry for monitoring trace element pollutants such as lead, therefore this technique was used to analyze samples for lead (IAEA 1985). American Public Health Association (APHA) standards where followed when analysing the hair samples.

2.5 Data Analysis

Data was analyzed using STATA 14.0. Haemoglobin levels were used to determine anaemia status using the cut-off points recommended by the WHO as defined above. Due to the small sample size, haemoglobin levels were analyzed as “normal” and “anaemic” instead of “normal”, “mild anaemia”, “moderate anaemia” and “severe anaemia”. Height, weight and age data was used to calculate BMI as an indicator of thinness as well as height-for-age as an indicator of stunting. The WHO 2007 Child Growth Standards macro package was applied in STATA to calculate the anthropometric data. Those with a z-score <-2 were classified as stunted. Demographic characteristics have been summarized in a table and a histogram has been used to depict distribution of haemoglobin and lead, respectively. To address the sub-aim and investigate the independent association between the categorical outcome anaemia and basic demographic characteristics or the continuous outcome haemoglobin and basic demographic characteristics, Fisher’s Exact test and linear regression was applied, respectively. A final logistic regression model examined the relationship between the predictor variables and anaemia as the binary (yes/no) outcome. A final multivariable regression model was used to analyze the association between the predictor variables and haemoglobin as the continuous outcome.

2.6 Ethical Considerations

The study protocols were approved by the IRB boards of both Duke Kunshan University in China as well as the University of Ruhuna in Sri Lanka. Consent forms were completed by parent(s) of all participants as well as participants between 12-14 years of age.

3. Results

Due to an unforeseen technical malfunction of the analytical equipment to be used for the processing of the samples, primary biological data have not yet been analyzed and the results will therefore focus on the variables obtained from the questionnaires and provide only a summary of the study population background characteristics and results for the sub-aim previously identified.

Table 1: Basic demographic characteristics of participants, anaemic and not anaemic combined

	Total (n=106)	%
Gender		
Male	19	17.9
Female	87	82
Age (years)		
Under 5	43	40.6
Over 5	63	59.4
Ethnicity		
Sinhala	96	90.6
Tamil	5	4.7
Muslim	5	4.7
BMI		
Underweight	71	66.9

Normal weight	30	28.3
Overweight	5	4.7
Stunting		
Yes	15	14.2
No	91	85.8

Due to some oversampling for lab analysis, there were a total of 106 participants of which 51 had anaemia and 55 did not. The basic characteristics of participants are displayed in Table 1. Most of the sample was ethnically Sinhalese, accounting for 90.6% of the total sample. Most of the children were female (82%) due to the technical difficulty of collecting hair samples from boys due to shortness of hair. 66.9% of sampled children were underweight and 14.2% were stunted. The participants age was fairly evenly distributed between under 5 years of age (40.6%) and over 5 years (59.4%). WHO haemoglobin cutoffs for anaemia status differed for children under and over 5. The more detailed categorization of anaemia according to WHO cut-off standards is displayed in Figure 1. Haemoglobin was normally distributed in the entire population as can be seen in Figure 2.

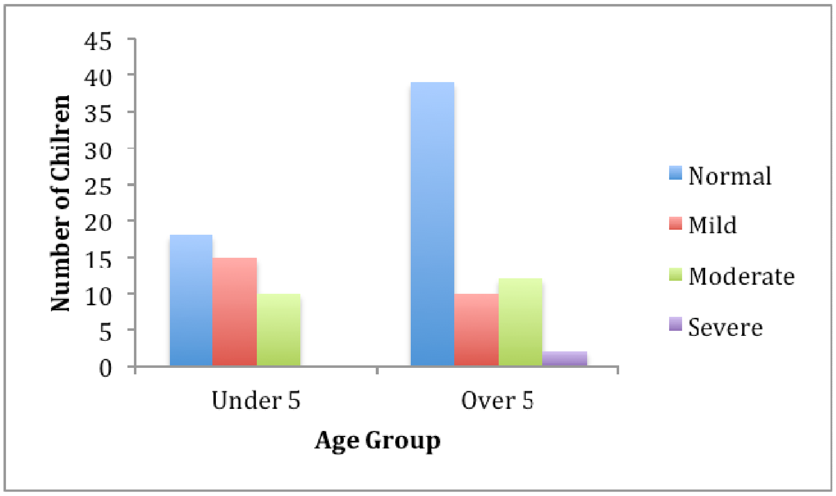

Figure 1: Distribution of anaemia by age group, applying WHO anaemia categories

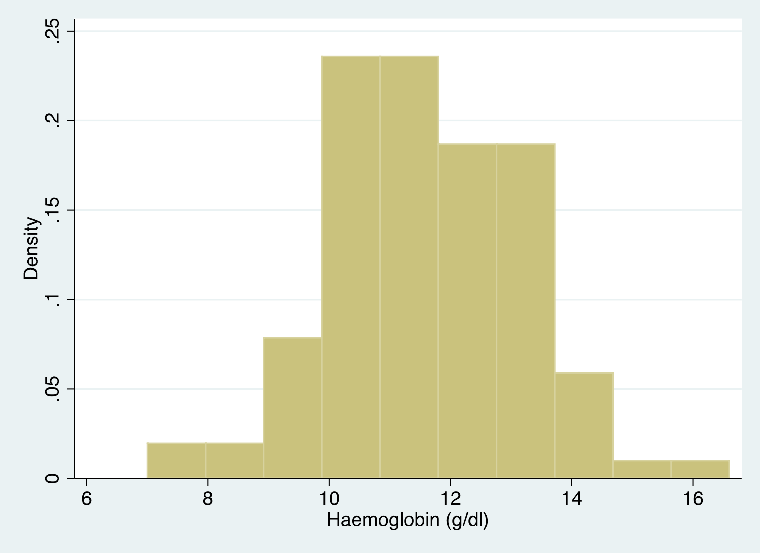

Figure 2: Distribution of haemoglobin among all participants

The mean haemoglobin by age seen in Table 2, was fairly similar across gender and age groups with the lowest in males under 5 (10.24 g/dl) and the highest in females over 5 (12.16 g/dl). A linear relationship between age and haemoglobin was found (Figure 3). Haemoglobin levels increased with age when combining girls and boys. Table 3 displays the independent association of predictor variables with haemoglobin and also with anaemia. The only variables significantly associated with having anaemia were male gender (p=0.003) and under 5 age (p=0.012). The only variables significantly associated with lower haemoglobin levels were also male gender (p=0.009) and under 5 age (p=0.0009).

Table 2: Distribution of mean haemoglobin concentration by age and gender

	N (%)	Hb (g/dl)	
Gender & Age		Mean	SD
Males: Under 5	10 (52.6%)	10.24	0.744
Over 5	9 (47.4%)	11.36	1.492
Females: Under 5	33 (37.9%)	11.25	1.430
Over 5	54 (62.1%)	12.16	1.615

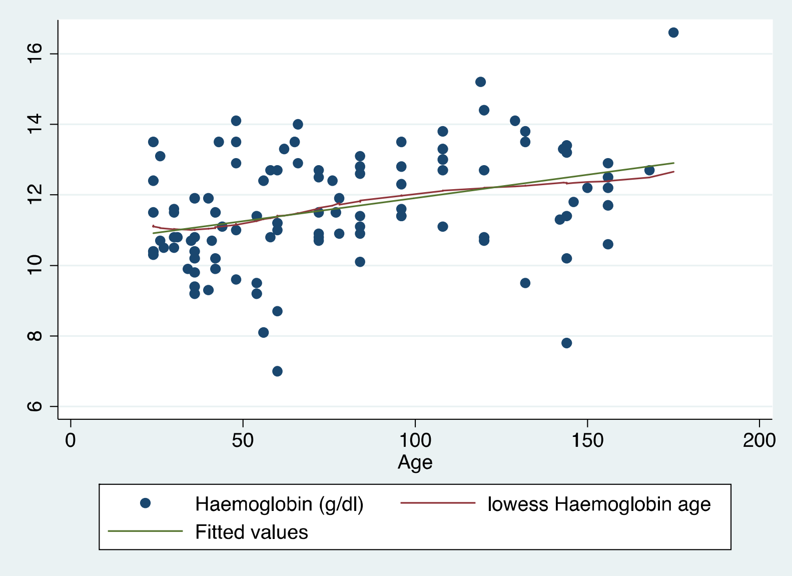

Figure 3: Scatter plot of haemoglobin and age with fitted values to show linear associations

Table 3: Individual relationship between population demographic characteristics and anaemia status and haemoglobin concentration

	Not anaemic	Anaemic	p value *	Haemoglobin (mean)	SD	p value **
Variables						
Gender						
Male	4	15 (78.9%)	0.005	10.76	1.262	0.009
Female	51	36 (41.4%)		11.81	1.602	
Age						
Under 5	16	27 (62.8%)	0.017	11.01	1.635	0.0009
Over 5	39	24 (38.1%)		12.04	1.612	
Ethnicity						
Sinhala	52	44 (45.8%)	0.286	11.71	1.577	0.111
Tamil	2	3 (60.0%)		11.4	1.69	

Muslim	1	4 (80.0%)		10.2	1.444	
BMI						
Underweight	36	35 (49.3%)	0.096	11.53	1.628	0.069
Normal	14	16 (53.3%)		11.59	1.411	
Overweight	5	0 (0.0%)		13.22	1.547	
Stunting						
Yes	6	9 (60%)	0.407	11.26	1.608	0.349
No	49	42 (46.2%)		11.68	1.494	

*Bivariate analyses, Fisher’s exact test

** Bivariate analyses, Linear regression

Table 4: Relationship between anaemia and population characteristics gender, age, BMI, stunting and ethnicity

Anaemia	N(%)	Odds Ratio	p value	95% CI
Gender				
Male	15 (78.9%)	Ref		
Female	36 (41.4%)	0.112	0.006	0.023-0.541
Age				
Under 5	27 (62.8%)	Ref		
Over 5	24 (38.1%)	0.478	0.106	0.196-1.170
BMI				
Underweight	35 (49.3%)	Ref		
Normal	16 (53.3%)	1.621	0.316	0.631-4.167
Stunting				
No	42 (46.2%)	Ref		
Yes	9 (60%)	1.630	0.432	0.482-5.513
Ethnicity				
Sinhala	44 (45.8%)	Ref		
Other	7 (70%)	5.941	0.114	0.650-54.281

* Logistic regression model, bivariate outcome anaemic/not anaemic

** Overweight children were not analyzed in this model as there were no anaemic overweight children in sample.

A logistic regression model was run to predict anaemia based on categorical variables: gender, age, BMI, stunting and ethnicity. Female children were significantly less likely to be anaemic (OR=0.122) and children over 5 were also less likely to be anaemic (OR=0.494). Children with normal BMI levels were actually more likely to be anaemic (OR=1.591) while overweight children were significantly less likely to be anaemic (OR=0.777). Children who were stunted, had a higher odds of being anaemic (OR=1.597) and children with any ethnicity other than Sinhala also had higher odds of being anaemic (OR=5.621).

Table 5: Relationship between haemoglobin and population characteristics gender, age, BMI, stunting and ethnicity

Haemoglobin (g/l)	Coefficient	Standard Error	p value	95% CI
Gender				
Male	Ref			
Female	0.932	0.377	0.015	0.183-1.68
Age				
Under 5	Ref			
Over 5	0.787	0.299	0.01	0.195-1.38
Ethnicity				
Sinhala	Ref			
Tamil	-1.613	0.68	0.020	-2.967- -0.264
Muslim	-0.305	0.684	0.657	-1.663-1.053
Stunting				

No	Ref			
Yes	-0.288	0.408	0.481	-1.097-0.521
BMI				
Underweight	Ref			
Normal	-0.121	0.322	0.707	-0.759-0.517
Overweight	1.783	0.705	0.013	0.383-3.183

* Multivariable regression, haemoglobin is continuous outcome (g/L)

A multivariable regression was run to predict haemoglobin based on gender, age, BMI, ethnicity and stunting. Only 18.44% of the change in haemoglobin could be explained by the model. It was found that higher haemoglobin counts were significantly predicted by female gender (Coeff=0.932, p=0.015) and age over 5 years (Coeff=0.787, p=0.01). Muslim children had lower haemoglobin than Sinhala children (Coeff= -0.305, p=0.657) and Tamil children had significantly lower haemoglobin than Sinhala children (Coeff= -1.613, p=0.02). Stunted children also had lower haemoglobin levels, although stunting did not contribute to the model significantly (Coeff= -0.288, p=0.481). Children of normal weight had lower haemoglobin than underweight children (Coeff= -0.121, p=0.707) while overweight children had significantly higher haemoglobin than underweight children (Coeff=1.783, p=0.013).

In the absence of primary data to support analyses relating to the primary aim of the study, similar studies have been reviewed. There is currently no research studying association between anaemia and lead in Sri Lanka, however such studies have been conducted elsewhere in the Asia region (Brázdová et al. 2014, Jain et al. 2005, Ahamed et al. 2007, Shah et al. 2010). Additionally, most studies measure blood lead as the exposure variable instead of hair lead levels. A study in India by Ahamed et al, found that children with blood lead levels >10µg/dl were 2.87 times as likely to have anaemia after adjusting for age, sex and area of residence.

Although we would not have established any causal pathway in this study, we anticipated a positive correlation between hair lead levels and anaemia as found in previous studies. A study in three countries belonging to Central Asian Republic found higher than expected lead levels in the hair of children hospitalized due to anaemia (Brázdová et al. 2014). While this study does not share a similar design and no statistically significant correlation was found between lead and anaemia, we would similarly expect to see higher lead levels in the hair of anaemic children.

Upon receiving the result from the analysis of hair samples, lead will be analyzed as a continuous variable in a multivariable regression model with anaemia as the categorical outcome. A linear association between hair lead and haemoglobin will be displayed visually in a scatter plot.

4. Discussion

While this study found lower haemoglobin in males than females, other studies of anaemia in Sri Lanka have found slightly different results. One study found no significant difference in the prevalence of anaemia between genders even within various ethnic groups (Amarasinghe et al. 2017), while another found a higher number of anaemia cases in females than males (Allen et al. 2017). Additionally, a study of children under 3 years in Brazil found that males were more likely to be anaemic (Zuffo et al. 2016). Anaemia in females is generally higher especially above an age when menstruation has begun, however since this study population is mostly under the age of 12, menstruation is not a factor contributing to anaemia here and this may explain lower than expected anaemia cases among females (Allen et al. 2017).

Number of anaemic children decreased with age, with only 38% of children over 5 having anaemia as opposed to 62.8% under 5, and a linearly increasing relationship between age and haemoglobin could be seen. A study in India similarly found that haemoglobin concentration increased in both girls and boys until approximately the age of 12. Above the age of 12, haemoglobin concentration in boys continued to rise whereas the haemoglobin concentration in girls fell. Large gender differences seen after the beginning of menarche in women indicate that the main cause of anaemia in that setting is iron deficiency (Alvarez-Uria et al. 2014). In this study, although most participants are female, almost all are under the age of 12, which explains the overall increase in haemoglobin with age.

Micronutrient deficiency in Sri Lanka has been described as a “hidden hunger” and is thought to be a more serious issue than energy deficiency (Jayatissa et al. 2015). Decreases in micronutrients have a relationship with the absorption of heavy metals and both can also cause anaemia independently or in unison (Hegazy et al. 2010). Iron deficiency and chronic lead exposure are thought to have a synergistic effect on lead levels in the body (Khan, Ansari and Khan 2011). The absorption pathway for iron in the gastrointestinal tract is through the divalent metal transporter-1 (DMT1). It has been theorized that absorption of lead occurs through this same receptor, therefore, an increase of lead absorption occurs during iron deficiency (Khan et al. 2011). A study by Khan et al found that children with iron deficiency had higher blood lead levels compared to children without iron deficiency across both the group “exposed” to lead and the control group.

Despite ongoing theories, there has been conflicting evidence regarding whether iron stores actually have a relationship with lead levels in the body. A study examining environmental lead contamination found that higher blood lead was associated with iron deficiency in children living in contaminated areas. A 1.7μg/dL increase in blood lead was seen in children with low serum ferritin as opposed to children with normal serum ferritin (Bradman et al. 2001). Another study recruiting children from the NHANES III in the US found no difference in iron status of children with low to moderate blood lead levels (Serwint et al. 1999). The interaction between lead exposure and iron deficiency should be studied further, but since data regarding serum ferritin has not been collected it is impossible to determine the role of iron deficiency in this study. In a subsequent study, it would be advisable to collect data regarding iron deficiency to understand it's true contribution to anaemia and any interaction with lead exposure.

Anaemia increases the absorption of heavy metals but conversely exposure to heavy metals also causes anaemia (Turgut et al. 2007). Due to this two-way interaction, the temporal order of causality may be difficult to determine. Since the outcome was ascertained before the exposure, temporality is not ascertained in this study. Additionally, the outcome variable of anaemia was obtained from medical records, where the timing of haemoglobin measurements varied amongst children. However, since lead is a continuous and accumulating exposure, in the case that the participant had anaemia at a previous time point, exposure to lead could have been a contributing factor regardless of exact anaemia status at the time of study enrolment.

While this study did not find a significant association between being underweight or stunting and anaemia or lower haemoglobin levels, interestingly children who were normal weight had lower haemoglobin levels and were more likely to be anaemic than underweight children. Other studies have generally found a higher risk of anaemia and lower haemoglobin levels in children who are underweight and/or stunted than children who have a normal weight. A study of undernutrition and anaemia in children under 5 years in India, found that underweight and stunted children had a 1.66 odds of having moderate to severe anaemia (Awasthi et al. 2003). In Sri Lanka, a study had similar findings with anaemic boys having a 3-fold higher risk of being underweight, although anaemic girls were found to have a 0.7-fold risk of being underweight (Hettiarachchi and Liyanage 2012). This study has an opposite finding, that children who have normal weight have lower haemoglobin levels and higher odds of being anaemic than underweight children. This difference may exist due to sampling variability and small sample size in this setting. As mentioned previously, micronutrient deficiency is still an issue in Sri Lanka. A report on the nutritional status in Sri Lanka reported that dietary diversity is low especially for certain food items such as fruits, vegetables, meat, fish and dairy (Rajapaksa et al. 2011, Jayatissa and Ranbanda 2006). Ongoing micronutrient deficiencies could be a contributing factor to anaemia even in children who are not underweight and may provide an explanation for the results seen in this study.

66.9% of children in this study were considered underweight with 73.7% of males and 65.5% females being underweight. While national prevalence from the National Nutrition and Micronutrient Survey was only reported to be 23.5% overall, other studies have reported figures as high as 65% (Jayatissa and Ranbanda 2006). Since the national survey included only children ages 6-59 months, this may account for some of the differences seen in underweight children. 14.2% of the study population was found to be stunted which is similar to the national prevalence in Sri Lanka in 2012 of 13.1% (Jayatissa et al. 2012). Stunting is strongly associated with socioeconomic status, and increases with impoverishment according to an analysis of DHS 2006/2007 data in Sri Lanka (Rannan-Eliya et al. 2013). In this setting data regarding socioeconomic status was not collected, therefore this association is not confirmed.

Few studies have analyzed hair lead levels, due to the lack of reference values and research in this area. A panel discussion by the Agency for Toxic Substances and Disease Registry (ATSDR) states that further research is needed to establish reference values (Harkins and Susten 2003). In the future, researchers need previously found lead values to be able to interpret whether results are higher or lower than expected and the primary data collected for this research will provide these values. While difficulties have been identified determining if contamination is endogenous or exogenous, (Pizzol, Thomsen and Andersen 2010) samples have been washed to remove any external contaminants and ensure that samples are reflective of internal dose. The hair lead data presented in a subsequent publication will be a reference for this region and can be used as a basis for future studies to determine hair lead trends in this region.

4.1 Limitations

Due to the difficulties surrounding participant recruitment described in the challenges (below), a higher proportion of boys with anaemia may have been recruited. While higher prevalence of anaemia has been observed in young boys than girls in other settings (Amarasinghe et al. 2017) which aligns with this study, future studies should ensure an unbiased recruitment of participants. Due to the variability in the timing of haemoglobin measurements, some outcome misclassification may have occurred, especially in instances where measurements were taken several months prior to study enrolment. Gaps in data exist regarding the distinction between anaemia, iron deficiency anaemia and iron deficiency without anaemia and the relationship between these conditions. In this study we were unable to collect serum ferritin data and therefore cannot differentiate between anaemia and iron deficiency anaemia. The WHO classifies anaemia according to age and gender. Cutoff levels vary slightly for children under 5 year, children 5-12 years and children 12-14 years. In this study, due to the small number of children over 12, age groups were only divided into under 5 years and over 5 years. In a larger study exact WHO cutoffs should be followed. Since this study only examines data from a very specific region within Sri Lanka, the findings will not be generalizable to other settings and there is limited external validity. Additionally, while measuring hair lead was the most practical and feasible option for this study, there has been some discussion regarding the validity of hair as a biomarker for exposure to toxic elements. Some studies have expressed concern whether hair lead levels may be reflective of the internal dose. Skröder et al. found that there was no association between hair lead concentrations and concentration of lead in erythrocytes and urine. In this study, concentration of lead increased with hair length, indicating that external contaminants contribute to concentration of contaminants (Skröder et al. 2017). This study has mitigated this issue by washing the hair samples to remove any external contamination. There is a lack of knowledge regarding the incorporation of lead into hair as well as lack of epidemiological data to predict health effects associated with hair lead concentrations. Further research is needing to provide reference ranges for interpreting results.

Multiple testing is an issue in studies analyzing many variables, testing one association after another for the same outcome variable, until a significant result is found. As the number of independent null hypotheses tested increases, the chance of a type I error also increases and the number of null hypotheses falsely rejected increases. There are statistical methods to correct for this, with the most conservative method being the Bonferroni Method which divides the usual significant p value of 0.05 by the number of individual tests conducted. Unfortunately, reducing type I error increases the chances of type II error occurring, so the chances of accepting the null hypothesis even when it should be rejected increases. A paper by Rothman suggests that actually no adjustment is needed for multiple comparison. The underlying theoretical premise for adjusting for multiple comparisons is the theory that "chance" primarily serves as the explanation for all observations. This assumes that when p value is set, all null hypotheses are true and any significant association is due to chance. However, if adjustments are made when not all null hypotheses are true, this results in weakening associations in the data (Rothman 1990). In the case of this research, literature already reveals that there are associations between the predictor variables gender, age, ethnicity, BMI and stunting and anaemia, therefore adjusting for multiple testing is not necessary or appropriate. Instead, findings have been discussed and examined based on previous literature and epidemiological evidence.

4.2 Challenges and Lessons Learned

This study has experienced numerous challenges especially related to the timeline. Recruitment of participants suffered from a significant setback of at least one month, due to an adenovirus outbreak in the study setting. Local populations avoided the hospital for some time due to fear of contagion, and the recruitment process was largely unsuccessful in the beginning of the recruitment period as a result. Recruitment was subsequently extended beyond the initial timeline of May to July, with the new recruitment period spanning from May to October. In addition, there was also some resistance from the parents of children during the process of hair sample collection. Some parents had voiced concerns with the local doctors regarding the aesthetic issues associated with cutting out patches of hair. This issue was resolved with the doctors in the hospital and more detailed explanations regarding the sample collection were given to the parents from there onward. Data collection was also hampered by the fact that it was logistically difficult to administer questionnaires and obtain biological samples in a crowded hospital setting with limited research personnel. In the future an alternative setting for data collection should be considered.

The process of data analysis was a great deal slower than anticipated due to some technical issues at the lab in Matara. The equipment used for lead analysis broke down and required specialized attention to be repaired, which set back the analysis of hair samples for a several months. There was a lack of trained personnel to repair the lab equipment and no other equipment was available in the country. Despite these setbacks, unexpected events are a part of fieldwork and it is important to learn to work around such issues with conceptual and methodological flexibility and strong teamwork. This study strongly reflects the realities of working in a setting with severely constrained resources, which was a valuable learning experience in itself. The limitations and capacity of the setting must be carefully considered when undertaking and designing research. Any further studies in this region should account for the technological capacities of local laboratories and seek to make alternative arrangements if feasibility is lacking.

4.3 Value of Study

In addition to being the first study in Sri Lanka examining the relationship between lead exposure and anaemia, this is also the first study in this setting to analyze lead in hair samples. This study will provide reference values for hair lead levels in this region, which will serve to inform future studies. The comparative design of this study is different from other studies analyzing hair lead and anaemia. The design of this study will have the added value that differences between anaemic and not anaemic groups can be studied. Since anaemia is still considered to be an issue in this region and current prevention practices have not eliminated the issue, it is important to conduct further research to adequately understand the aetiology and effective prevention strategies. Thus, this study can have important policy implications regarding the contamination of the environment by heavy metals and the potential health impacts. Further research is needed to understand the relationship, identify best practice strategies and raise awareness regarding the contribution of heavy metal exposure to anaemia.

5. Conclusion

Anaemia is an ongoing issue in Sri Lanka, however the basic demographic characteristics assessed in this study were not strongly associated with anaemia. While anaemia is multi-causal, it is expected that exposure to lead is associated with lower haemoglobin levels and may be a contributing factor. Hair lead levels will be analyzed as soon as the technical capacity of the local lab is restored, and findings will be written up for a subsequent journal publication. The challenges described here provide important lessons and will serve to inform future research in this region.

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