博士論文

# Impact of Prenatal Heavy Metals Exposure on Birth Outcomes and Newborns Leucocytes Telomere Length in Myanmar

（妊娠期における重金属暴露が出生アウトカム及び新生児の白血球テロメア長に与える影響：ミャンマーにおける研究）

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TABLE OF CONTENTS

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AOR | Adjusted Odds Ratio |
| ATL | Alternative Telomere Lengthening |
| CI | Confidence Interval |
| CV | Coefficients of Variation |
| DL | Detection Limit |
| EDTA | Ethylene-Diamine-Tetra-Acetic Acid |
| GM | Geometric Mean |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| IQR | Interquartile Range |
| NIES | National Institute for Environmental Studies |
| NIST | National Institute of Standards and Technology |
| OR | Odds Ratio |
| PCR | Polymerase Chain Reaction |
| ROS | Reactive Oxygen Species |
| SD | Standard Deviation |
| TL | Telomere Length |
| WHO | World Health Organization |

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

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## ABSTRACT

**Introduction**: Arsenic, cadmium and lead are well-known environmental contaminants and their toxicity at low concentrations is a target of scientific concern. Through trans-placental exposure, these metals can accumulate in fetal tissues, extending the risk of fetal toxicity. Meanwhile, heavy metals exposure appears to modify telomere length (TL), which may predispose a person to adverse health risks. Information on newborn TL is important because TL later in life is mainly influenced by the TL at birth, meaning that having a short or long TL is greatly defined before adulthood. However, the determinants of TL at birth were poorly explained. This study aimed to examine the extent of heavy metals contamination among a Myanmar population. This study also identified the potential effects of prenatal heavy metals exposure on birth outcomes among pregnant women and newborns in Myanmar. In addition, this study determined whether prenatal heavy metals exposure has an impact on newborn leucocyte TL. For a better understanding of the etiologic pathway, this study also determined whether heavy metals-induced TL shortening triggers the occurrence of adverse birth outcomes.

**Methods**: A birth-cohort study was conducted among 419 pregnant women in Myanmar. At their first visit, face-to-face interviews were performed, and maternal spot urine was collected. Cord blood samples were collected at the time of delivery during follow-up. Urinary arsenic, cadmium, lead and selenium concentrations were measured by inductively coupled plasma mass spectrometry (ICP-MS) and adjusted for creatinine. TL was measured by quantitative real-time polymerase chain reaction (PCR), and relative TL was calculated as the ratio of telomere repeats product to a single-copy gene (T/S ratio). We examined the prenatal exposure of arsenic, cadmium and lead and their associations with adverse birth outcomes and newborn TL using multivariable logistic and linear regression analysis, respectively. Later, we determined whether a shorter TL was associated with risks of adverse birth outcomes using logistic regressing analysis.

**Results**: The median values of adjusted urinary arsenic, cadmium, selenium and lead concentrations were 74.2, 0.9, 22.6 and 1.8 μg/g creatinine, respectively. Prenatal cadmium exposure was positively associated with risk of low birth weight (adjusted odds ratio (AOR) = 4.79, 95% confidence interval (CI): 1.25, 18.37, $p$ = 0.022) after adjusting for maternal age, maternal education, the baby's sex, smoking status, primigravida, antenatal visits and selenium concentration. However, maternal heavy metals concentration was not significantly associated with preterm delivery. There was an independent inverse association between prenatal arsenic (lowest vs highest quartile, coefficient = - 0.13, 95% CI: - 0.22, - 0.03, $p$ = 0.002), cadmium (lowest vs highest quartile, coefficient = - 0.19, 95% CI: - 0.30, - 0.08, $p$ < 0.001), and lead exposure (lowest vs highest quartile, coefficient = - 0.11, 95% CI: - 0.20, - 0.02, $p$ = 0.020) and newborn TL, even after adjusting for maternal age, education, ethnicity, smoking status,

parity, gestational age, mode of delivery, the baby's sex, birth weight and selenium concentration. Newborn TL was not significantly associated with any adverse birth outcomes.

**Conclusions**: The present study determined that Myanmar mothers are highly exposed to cadmium. Prenatal maternal cadmium exposure was associated with an increased risk of low birth weight. This is also the first study to determine the impact of prenatal heavy metals exposure on newborn TL. The present study also identified that arsenic, cadmium and lead exposure could shorten TL, even *in utero* exposure. Since the TL at birth could predict the TL later in life, future public health measures should integrate interventions to reduce heavy metals contamination, with special emphasis on pregnant women. However, the risk of adverse birth outcomes was not associated with newborn leucocyte TL.

*Keywords: heavy metals, birth outcome, telomere length, newborn, Myanmar*

# Chapter 1: Introduction

## *1.1 Heavy Metals Exposure and Health*

Toxic heavy metals such as arsenic, cadmium and lead are naturally or anthropogenically dispersed in the environment [1, 2]. They may enter the human body through dermal contact, oral and/or inhalational routes [1]. Once entered, they are deposited into the human tissues with lengthy half-lives and subsequently, may pose several health hazards [1-3]. The state of toxicity in the body is influenced not only by exposure to high concentrations but also by the gradual accumulation of lower concentrations of heavy metals when the detoxification process takes place at a much slower rate than accumulation [1].

Arsenic is a well-known toxic heavy metal and recognized as a human carcinogen [4, 5]. It naturally occurs in groundwater and its contamination has become a severe public health concern, especially when it is the primary source of drinking water for people living in contaminated areas [4, 6]. Worldwide, approximately 200 million people are suffering from chronic arsenic toxicity through arsenic-contaminated drinking water [4, 6, 7]. India and Bangladesh are particularly notable for their high arsenic contamination in groundwater with a large affected population size and relatively severe health effects [4]. Several countries across Southeast Asia were recently identified for groundwater arsenic contamination including Indonesia, Vietnam, Thailand, Cambodia, Laos, and Myanmar [4, 8]. Arsenic toxicity may result in both acute and chronic poisoning mostly through ingestion [9]. Several studies have proved that long-term arsenic exposure has adverse impacts on human health; causing skin

lesions, cardiovascular diseases, neurological complications, reproductive disorders, respiratory disorders, malignant diseases and non-malignant diseases [10-15]. Furthermore, arsenic passes the placental barrier from the mother to the fetus, further extending the health consequences [16].

Cadmium is a ubiquitous element in the environment and is toxic even at low concentrations [1, 17]. The 'itai-itai' disease in Japan brought the world's attention to the dangers of environmental cadmium exposure and has led to numerous reports on the health risks of cadmium among human populations [18, 19]. The general population can be exposed to cadmium mostly through the diet, tobacco smoking and industrial activities [18]. Cadmium exposure primarily affects the kidney, liver and bones; however, growing evidence indicates that cadmium exposure, even at a low concentration, also has a carcinogenic effect and is associated with risks of chronic disease [18, 20-22]. Moreover, cadmium can extend the health risks to the fetus and greatly accumulate in the placenta [20, 23]. Meanwhile, it can inhibit the synthesis of hormones, such as progestogens, testosterone, leptin, and thyroid hormones, by acting as an endocrine disruptor which eventually has effects later in life [24, 25].

Lead is also an environmental contaminant that is naturally found at low concentrations in the earth's crust; perhaps many industrial activities have predisposed its widespread occurrence [1, 26-28]. The general population is mostly exposed to lead through inhalation or ingestion, equally [1, 2]. The degree of lead exposure varies broadly across regions depending on urbanization, industrialization and lifestyle [27, 28]. Environmental exposure to lead poses a major health challenge, particularly, its neurotoxic effects consistently threaten developing children [28-30]. Lead exposure can

also contribute to the etiological backgrounds of many diseases including renal, neurological, haematological, and cardiovascular diseases [28]. It is reported that inorganic lead exhibits mutagenic, clastogenic and carcinogenic properties [26]. Like arsenic and cadmium, lead can also easily pass through the placenta and accumulate in fetal tissues [3].

Selenium naturally exists in the environment and is unevenly distributed over the earth's surface [31]. Humans consume selenium from foods, and the selenium content of foods varies depending on the selenium content of soil where the foods are produced [31-33]. Selenium is one of the essential nutrients for the detoxification of oxidative stress, owing to the active site of glutathione peroxidase [32, 34, 35]. The antioxidant effect of selenium plays a significant role in anti-carcinogenesis since it limits DNA damage induced by oxidative stress [31, 32]. In addition, high selenium in the form of selenoenzymes or selenoproteins can prevent the generation of oxidized low-density lipoprotein, thereby regulating the plasma cholesterol level [32]. It was previously reported that selenium deficiency can predispose humans to serious health problems such as Keshan disease, Kashin-Beck disease, carcinogenicity, and cardiovascular diseases [31]. In contrast, an estimated safe oral intake of selenium is 5 µg per day per body weight (kg) for adults, perhaps it differs across regions [31]. Although sensitive biochemical indicators of selenium overdose are limited, it has been reported that both animals and humans who consume more than a safe level of selenium can exhibit hair and nail lesions, peripheral neuropathy and reproductive problems [31, 33]. Moreover, previous studies have confirmed that selenium also has maternal-fetal transfer [36-38].

*1.2 Heavy Metals Exposure and Maternal and Newborn's Health*

Pregnant women and their fetuses are more vulnerable to the adverse effects of the exposure to environmental toxic substances [16, 39, 40]. If exposure happens *in-utero*, the effect could cause a negative impact in early childhood and later in life; through permanent structural and functional changes [41]. Although the placenta may act as a selective transporter that prevents the passage of potentially toxic substances to the developing fetus, some environmental contaminants can freely or partially cross the placental barrier [37]. In particular, arsenic, cadmium and lead are well-known environmental heavy metals that could extend the health risk to the fetus even at a low concentration through trans-placental circulation [36, 39, 42, 43].

The toxicologic effects of heavy metals could alter the prenatal stage of human development which is a critical period of fetal cell division and differentiation [41]. For example, prenatal cadmium exposure could impair steroidogenesis which leads to suboptimal fetal growth and development [24]. Lead exposure could interfere with calcium deposition into the bone, resulting in decreased fetal bone growth [44]. Arsenic exposure during pregnancy may also contribute to placental insufficiencies that could lead to intrauterine growth retardation by inducing oxidative stress [45].

The associations between prenatal exposure to environmental heavy metals and adverse birth outcomes have been examined in varying degrees over the last decades. In many studies, prenatal arsenic, cadmium and lead exposure were inversely associated with the anthropometric measures of newborns such as birth weight, birth length, and head circumference [43, 46-50]. Moreover, exposure to these metals also increased the risk of preterm delivery [51-53]. Inorganic arsenic exposure during pregnancy was also

positively associated with the risks of stillbirth and miscarriage [54]. Although a definitive explanation for heavy metals-induced adverse birth outcomes is lacking, these metals are known to be involved in oxidative stress induction, hormone interactions and direct toxicity which could lead to defective placentation [24, 37, 45]. Since adverse birth outcomes could increase the lifelong mortality risk later in life, there has been increased awareness with regard to predicting the potential health impact of prenatal heavy metals exposure on birth outcomes [55, 56].

### *1.3 Biological Significance of Telomeres*

#### *1.3.1 Telomere Biology and its Role*

Telomeres are repeated sequences of oligomers (TTAGGG) located at the human chromosomal ends. They prevent chromosomal damage by protecting against chromosome-chromosome fusions and degradation, thereby maintaining genomic integrity [57-59]. Telomere length (TL) is largely maintained by the telomerase RNA protein complex and the shelterin proteins whose levels are regulated by human telomerase reverse transcriptase (hTERT) and telomerase RNA genes (hTR) [60-64]. Telomeric DNA is more sensitive to oxidative stress due to its guanine-rich sequences [65, 66]. In addition to direct attack by reactive oxygen species (ROS), the DNA repair response is less competent in telomeric DNA than in the majority of other genomic DNAs [65]. This is because telomere-related proteins can interfere with the cellular responses to breaks on telomeric DNA strands by blocking access to DNA repair enzymes [65, 67, 68].

Telomere shortening occurs in normal somatic cells following each cell division [62, 64]. Once they reach a certain minimum length, cellular apoptosis occurs because of chromosomal end fusion [63, 64]. Based on existing studies, if cellular apoptosis does not occur at the critical TL, cells continue to divide due to activation of telomerases [57, 60, 62]. Telomerases add the telomere DNA sequences (TTAGGG repeats) to prevent chromosomal fusion, resulting in chromosomal instability [62]. Dysfunctional telomeres and telomerase activity may, in turn lead to genomic instability and malignancy [61, 69, 70].

### *1.3.2 Factors Associated with TL and Related Health Risks*

Inter-individual variation in TL can be mostly explained by variation at birth, although it also depends on TL attrition rate later in life [66, 71-74]. However, overall TL attrition during adulthood is relatively small, meaning that most adults maintain a fixed ranking and tracking of TL over their lifetime [71]. In relation to this, a Danish twin study estimated that the heritability of TL at baseline was 64% (95% confidence interval (CI): 0.39, 0.83) with significant shared environmental effects (estimate = 0.22, 95% CI: 0.06, 0.49) [74]. The intrauterine environment is potentially plausible for newborn TL modification perhaps via fetal programming cascade [75]. Meanwhile, the TL attrition rate depends on telomeric DNA susceptibility and responses to the intrinsic and/or extrinsic stressors and telomerase enzyme activities [75]. It has been established that TL is correlated with aging, exposure to genotoxic agents, oxidative stress and a wide range of environmental factors [64, 76-78]. Many factors such as obesity, smoking and environmental heavy metals exposure could lead to oxidative stress which in turn

accelerates telomere shortening [79-81]. Furthermore, these factors are closely associated with inflammatory responses, particularly the production of cytokines that could activate telomerase enzymes, resulting in TL shortening [82-85]. However, it is difficult to establish the causal relationship between such factors and TL because TL alteration could be a direct or indirect systemic effect of a response mechanism.

Genetic and epigenetic modifications are also associated with telomere maintenance. Polymorphisms in telomere-related genes, particularly hTERT genes are strongly associated with telomere instability and increased risks of various cancers [86]. Moreover, epigenetic regulations, both global DNA methylation and methylation status of subtelomeric regions, are involved in telomere dynamics [87, 88]. These epigenetic modifications could determine the expression of telomere-related genes which are crucial in telomere maintenance.

An increasing body of evidence has indicated associations between TL and pathophysiological outcomes later in life, such as aging, cardiometabolic diseases and malignancies [70, 86, 89, 90]. A recent meta-analysis comparing short TL to long TL subjects showed that the relative risk of cardiovascular diseases was 1.54 (95% CI: 1.30, 1.83) [90]. In another study, a shorter TL was found to be significantly associated with an increased metabolic risk profile for high-density lipoprotein ($\beta$ = - 0.016, $p$ = 0.05), triglycerides ($\beta$ = 0.038, $p < 0.001$), waist circumference ($\beta$ = 0.647, p = 0.007), and fasting blood glucose level ($\beta$ = 0.011, $p < 0.001$) [91]. In addition, telomere shortening; or lengthening increases the likelihood of malignancies. Therefore, perhaps TL differs between the types and stages of cancers. For example, telomere shortening is associated with an increased risk of bladder, gastric and oesophageal cancers [92-94], while

telomere lengthening increases the risk of melanoma, breast and lung cancers [95-97]. Nevertheless, it could be hypothesized that TL alterations could serve as a biomarker of a disease or risk; and thus, the assessment of TL could be useful in biomonitoring health risks.

### *1.4 Heavy Metals Exposure and TL*

#### *1.4.1 Arsenic Exposure and TL*

The impact of environmental and occupational arsenic exposure on TL has been increasingly concerning. An *in vitro* study on human cord blood cells stated that the TL increased upon exposure to a low concentration of inorganic arsenic (0.001 µM) after both 24 hours and 7 days of treatment, while the TL shortened at a high concentration of 1 µM after 7 days in culture [98]. Another study on human cell lines also supported the idea that telomerase activity induced by arsenite concentration at 0.1 - 1 µM resulted in telomere elongation, whereas a high concentration (more than 1-40 µM) shortened the TL [99]. A prospective cohort study in Bangladesh among 167 participants showed that a longer TL was highly prevalent among participants of a high arsenic exposure group (urinary arsenic > 339 µg/g) [100]. Another study in India also demonstrated that a longer TL was found among an arsenic-exposed population with skin lesions than in the control group (odds ratio (OR) 13.75; 95% CI: 5.66, 33.41) [101]. However, a study conducted among healthy subjects in Italy revealed an inverse association between arsenic exposure and TL ($\beta = -0.23$, $p = 0.08$) [102]. A cross-sectional study conducted in Northern Argentina found no association between urinary arsenic concentration

(median value = 230 µg/L) and TL [103]. Based on the existing studies, it is convincible that arsenic has a dose-response effect on TL and telomerase activity.

Without a doubt, arsenic is considered genotoxic since it induces oxidative stress; and alters DNA repair and DNA methylation patterns [104-106]. Thus, these mechanisms could potentially affect TL maintenance [68]. So far, many studies have postulated several possible underlying mechanisms of arsenic-induced TL alterations. Arsenic involved in telomere shortening through the mediation of arsenic-induced apoptosis, while it is also possible that arsenic-induced oxidative stress directly damages the telomeres and telomere-related proteins [79]. Since telomerase activation plays a crucial role in telomere maintenance [60, 62], the effect of arsenic on hTERT gene expression has also been investigated. A study conducted among the villagers of inner Mongolia reported that hTERT expression was highly associated with arsenic exposure both *in vivo* and *in vitro* [107]. In contrast, a case-control study in India found that telomere lengthening was independent on telomerases, and was possibly due to the upregulation of shelterin complex proteins such as TRF1 and TRF2, which trigger "alternative telomere lengthening" (ATL) [101]. Another study among a Bangladesh population also elucidated that arsenic exposure was correlated with the expression of other telomere-related genes such as WRC, TERF2, DKC1, TERF2IP, and OBFCI. The same study also suggested that alterations in the expression of these genes may cause telomere de-protection, contributing to the activation of ATL [100].

### *1.4.2 Cadmium Exposure and TL*

Environmental and occupational exposure to cadmium is associated with TL alterations. A handful of recent studies have explored the effect of cadmium on TL. *In vitro* studies of mouse embryonic stem cells showed that telomere shortening was triggered by chronic exposure to low-dose cigarette smoke (0.02 mg/mL) or cadmium (5-10 μM) [108, 109]. Regarding human studies, a population-based cross-sectional study in the U.S. reported that a higher cadmium concentration in both the blood (mean blood cadmium = 0.44 μg/dL) and urine (mean urinary cadmium = 0.28 μg/dL) was significantly associated with a shorter TL (β = - 5.54; 95% CI: - 0.42, - 0.47 for blood and β = - 4.50; 95% CI: - 8.79, - 0.20 for urine) [110]. Similarly, a cross-sectional study among Nepalese adolescents also reported that the urinary cadmium concentration (geometric mean = 0.19 μg/L) had a significant negative association with salivary TL (β = - 0.24; 95% CI - 0.42, - 0.07) [111]. Likewise, a concentration of 0.09 μg/dL cadmium initiated the placental telomere shortening (r = - 0.138, $p$ = 0.013) among a population in an electronic recycling waste town of China [23].

Possible mechanisms of cadmium-induced telomere mutagenicity have been proposed in previous studies. Since telomeres are notably sensitive to oxidative stress, cadmium could disturb the oxidative stress response by promoting the production of ROS [65, 112, 113]. Cadmium may also accelerate telomere shortening by stimulating the production of inflammatory chemicals, particularly cytokines [83, 84]. Furthermore, cadmium mimics the action of divalent metallic ions such as zinc which could interfere with the DNA repair system [114, 115]. Through these processes, cadmium may impact TL maintenance.

### *1.4.3 Lead Exposure and TL*

TL could also be modified by environmental lead exposure since inorganic lead also exhibits mutagenic, clastogenic and carcinogenic properties [26]. The effects of lead exposure on TL have been examined in several studies. A case-control study conducted among male lead smelters in Poland reported that telomere shortening was associated with a higher blood lead level (mean blood lead level was 33 µg/dL for the exposed group and 2.2 µg/dL for the control group) [116]. Another study conducted in the southern part of Poland also showed that children had a shorter TL ($\beta$ = - 0.13; 95% CI - 0.23, - 0.02) at the blood lead level of $\geq$ 3.2 µg/dL [78]. Similarly, a cross-sectional study among Chinese battery plant workers reported a strong negative correlation between peripheral blood TL and blood lead level (r = - 0.70, $p < 0.0001$) [117]. Conversely, blood lead level was not significantly associated with the relative TL in a population-based study using national health and nutrition examination survey data of the U.S. from 1999-2002 (mean blood lead level was 1.67 µg/dL) [110].

Researchers have speculated about the underlying molecular and biological mechanisms of lead-induced genotoxicity. Lead could induce genotoxicity through indirect pathways due to oxidative stress or DNA epigenetic modifications [1, 118]. Moreover, it may provoke TL alternations by interfering with chromatin organization, and causes nuclear membrane impairment, which is crucial for telomere stability [119, 120]. Lead can also replace calcium, zinc and/or magnesium in enzymes through the ionic mechanism of its toxicity, which can ultimately interfere with various biological processes including DNA processing and repair [26, 121].

### *1.4.4 Selenium Exposure and TL*

Selenium is considered an antioxidant and the nutritional level of selenium has an anticarcinogenic effect [31, 32]. Despite its antioxidant effect, epidemiological studies on the associations between selenium level and TL among human populations are limited. An *in vitro* study on human hepatocyte cell lines demonstrated that the TL of hepatocyte L-02 cells was significantly longer at 0.5 and 2.5 μmol/L sodium selenite [122]. Another study reported that at doses of 2.5, 5.0 and 10 μmol/kg selenium, the telomerase activity of rat hepatocytes increased significantly, supporting the mechanism of telomerase-dependent TL elongation [123]. Another study on yeast cells showed that selenium-treated cells had a significantly longer TL than control cells ($p$ = 0.031). This study also demonstrated that both lead and selenium-treated cells had a longer TL than lead alone treated cells, indicating that selenium can repair the damage by the lead to some degrees [124]. The antioxidant effect of selenium may involve in protecting or repairing of the TL, thereby decelerating telomere aging. It is worth investigating how selenium acts and mediates the effects of other metals on TL among human populations.

## *1.5 TL and Adverse Birth Outcomes*

TL shortening could be associated with the pathophysiology of adverse pregnancy outcomes, such as low birth weight and preterm delivery [125-127]. Placental cell aging is a physiological adaptive response of rapid cell division during pregnancy [125], and TL could be considered as a biomarker of cellular aging [58, 65-67]. Premature placental aging happens when the intrauterine environment becomes

hostile due to intrinsic or extrinsic chemical contaminants, leading to placental dysfunction [125]. Placental dysfunction, particularly; decreased placental growth and restricted nutriment supply to the developing fetus lead to intrauterine growth restriction [125, 127]. In addition, senescent cells may induce the aging process of adjacent cells by secreting high levels of inflammatory cytokines, metalloproteinases and epithelial growth factors [128]. As a consequence, aging and systemic inflammation may also provoke preterm delivery [125]. Hence, cumulative cell damage, shorten TL, may be closely associated with low birth weight and preterm birth.

Previous studies have reported that a shorter TL was associated with preterm, preterm premature rupture of membranes (pPROM), stillbirth and fetal growth restriction [127, 129-133]. A retrospective study in the Netherlands found that individuals born preterm had a significantly shorter TL than individuals born at normal term ($p = 0.003$) [133]. In contrast, in a comparative study of leucocyte TL in three groups of pPROM, preterm with intact membranes and normal full-term newborns, the TL was not significantly different between pPROM and normal full-term newborns ($p = 0.31$); however, preterm newborns had a significantly longer TL than pPROM ($p = 0.05$) and normal-term ($p < 0.01$) newborns [129]. Another study in Italy also reported that placental TL shortening was found among the stillbirth group ($p < 0.001$), suggesting that premature aging of the placenta may contribute to the etiology of stillbirth [131]. Similarly, the placental TL was significantly shorter among the newborns with fetal growth restriction compared to control group in a U.S. study ( $p < 0.001$) [127].

Telomerase activity may also play an important role in TL shortening in relation to adverse birth outcomes. A previous review reported that complicated pregnancies such as preeclampsia and/or fetal growth restriction had decreased or no telomerase activity, leading to shortening of the TL [126]. Another study in the U.S also demonstrated that higher expression of telomere-induced senescence markers such as elongation factor 1 alpha, p 21 and p16 were found among the fetal growth restriction group ($p < 0.01$), indicating that cellular senescence is more frequent among this group that may suppress placental telomerase activity [127].

Heavy metals-induced TL shortening may be an intermediate molecular cause of fetal stress and placental apoptosis which leads to adverse birth outcomes. Although a definitive explanation for the mechanism of heavy metals-induced adverse birth outcomes is not clear, heavy metals are involved in oxidative stress induction, hormone interactions and direct toxicity which could lead to defective placentation [16, 24, 40, 49]. Meanwhile, TL shortening is a biomarker of cumulative oxidative stress [65]. As mentioned above, there is an increasing evidence for associations between a shorter TL and adverse birth outcomes [127, 129-133]. Taken together, it is possible that TL shortening could be a proxy of placental dysfunction which triggers adverse birth outcomes.

### *1.6 Justification*

Heavy metals exposure is likely to modify the TL, which may be associated with adverse health risks. However, available information regarding the effect of

environmental heavy metals exposure on TL is controversial and limited. Previous studies have investigated the effect of heavy metals exposure on TL and telomerase gene expression; nevertheless, those studies focused only on adult or adolescent populations [100, 101, 103, 110, 111]. In the context of TL, the response to oxidative stress is different, even between mother and fetus [129]. Although the trans-placental transmission of some heavy metals, such as arsenic, cadmium and lead has been investigated [36, 39, 42, 43], so far, only one *in vitro* study had described the associations between prenatal arsenic exposure and newborn TL [98]. Therefore, it is important to pay attention to whether in-utero exposure to heavy metals could extend the effect to newborn TL.

Knowledge of newborn TL is relevant because the TL later in life is mainly influenced by the TL at birth, meaning that having a short or long TL is greatly defined before adulthood [71, 74]. So far, only a handful of studies have identified predictors of newborn TL [134-139]. For example, younger paternal age ($\beta = 0.016$, 95% CI: 0.004, 0.028, $p = 0.008$), higher maternal pre-pregnancy body mass index ($\beta = -0.52$, 95% CI: - 0.85, - 0.20, $p = 0.002$), and lower maternal education (Spearman's rho = 0.36, $p < 0.01$) are independent predictors of a shorter newborn TL [134, 138, 139]. Furthermore, maternal pregnancy-related psychological stress was negatively associated with newborn TL among a Pennsylvanian population ($\beta = -0.099$, 95% CI: - 0.197, - 0.002, $p = 0.047$) [136]. A recent prospective cohort study in Belgium also determined that high prenatal $PM_{2.5}$ (particulate matter $\leq$ 2.5 microns in diameter) exposure was correlated with a shorter newborn TL (8.8%, 95% CI: - 14.1%, - 3.1%) [135]. Since *in utero* exposure effects are closely correlated with early-life developmental effects and

ongoing health risks, partly through epigenetic modifications [140], it is crucial to identify the factors that influence TL at birth for preventive measures against human disease morbidity and mortality later in life.

Adverse birth outcomes such as low birth weight and preterm delivery, are closely associated with various lifelong mortality and morbidity risks [56, 141]. Generally, low birth weight babies are at increased risks of mortality, morbidity and disability since it is correlated with poor growth in childhood and an increased incidence of many diseases such as hypertension, cardiovascular diseases and type 2 diabetes mellitus in adulthood [56]. According to the World Health Organization (WHO), more than 15% of newborns are born with a birth weight less than 2500 g, with the developing countries accounting for more than 95% of them [56]. Preterm delivery is another major determinant of neonatal mortality and morbidity [142]. A study of 4 million early neonatal deaths over 193 countries reported that 28% of neonatal deaths were either directly or indirectly due to preterm delivery [143]. A recent systematic analysis showed that Southeast Asia accounted for the highest number of preterm birth rates in 2010, and estimated that 13.6% were born preterm [141]. Moreover, the associations between prenatal heavy metals exposure and adverse birth outcomes have been identified in many previous studies [43, 46-50]. Better understanding of the etiologic pathway between prenatal heavy metals exposure and adverse outcomes among a Myanmar population is relevant for preventive measures.

Meanwhile, a number of studies have demonstrated that a shorter TL is correlated with adverse birth outcomes such as stillbirth, preterm delivery and fetal growth restriction [127, 129-133]. Unfavorable conditions *in utero* can trigger oxidative

stress which, in turn causes placental insufficiency, leading to adverse birth outcomes [129, 144]. Consequently, adverse birth outcomes could alter TL homeostasis and accelerate TL shortening or vice versa [129, 131, 144]. The earlier TL shortening happens, the faster the intrinsic organs age, which is closely related to cellular dysfunction and disease susceptibility [126]. Therefore, it is important to understand the mechanistic pathway between TL homeostasis during fetal development and adverse birth outcomes.

To prevent waterborne infectious diseases, groundwater (from deep well) has been substituted as the source of drinking water in Myanmar. However, toxic heavy metals contamination in groundwater may increase the risk of many chronic diseases [145]. According to the World Bank Policy Report 2005, an estimated 3.4 million people were at risk of arsenic contamination mainly through ingestion in Myanmar [146]. The southern and central regions of Myanmar are confirmed to be highly contaminated with arsenic in the groundwater [145, 147-149]. High arsenic concentration in the groundwater has been predicted in the southern part of the country, the Ayeyarwady Region, based on the local hydrogeology and climate; reductive dissolution of iron oxyhydroxides in groundwater was assumed [147]. According to the previous national reports, in the Ayeyarwady Region, of total 123,964 drinking water samples; 29.18% are above the WHO standard for an arsenic concentration of 10 μg/L, and 8.19% exceed the arsenic concentration of 50 μg/L [148, 149].

Evidences regarding the other heavy metals contamination is limited in Myanmar. As mentioned previously, diet (rice in particular), tobacco smoking, and occupational exposure are the major sources of cadmium exposure in general [18]. In

Myanmar, only few women smoke, and occupational exposure in possible only in the regions where mining is conducted. Diet may be a major source of cadmium exposure since Myanmar people usually consume large amount of rice in their daily life. For lead, occupational activities such as mining, and battery industries are potential sources of exposure. In fact, a previous study conducted in central Myanmar reported that of 18 water samples, none of them was above the WHO standards of lead concentration (10 μg/L), and cadmium concentration was below the detection limit of 1 μg/L [145]. Little is known about the extent of heavy metals exposure among Myanmar population; assessment of exposure using biological samples is necessary.

Without a doubt, confirmed arsenic contamination is consistently threatening the health of Myanmar people. A previous study in central Myanmar reported that increased arterial blood pressure and low brachial index were found among those with a nail arsenic level higher than 0.09 μg/g [150]. Additionally, among the populations in the Ayeyarwady region, a negative association was found between 2-h creatinine clearance and a serum arsenic concentration of more than 0.008 μg/L, indicating that chronic arsenic exposure may affect renal glomerular function [151]. Despite significant arsenic health concerns and potential contamination of other heavy metals in Myanmar [145, 147], an exposure assessment using biological samples has rarely been conducted among a Myanmar population.

Myanmar is still on the way of progress in reducing neonatal mortality. As reported by the Myanmar Demographic and Health Survey 2015-16, the estimated infant mortality rate was 40 deaths per 1,000 live births, and more than 60% of deaths occurred during the first month [152]. According to the WHO, in Myanmar, the

estimated number of low birth weight babies was 179 per 1,000 live births in 2000 and the preterm birth rate was 12 per 100 live births in 2010 [56, 141]. In addition to investing in maternal and neonatal healthcare services, a wider range of underlying etiologic factors should be examined. Although heavy metals contamination has been confirmed, no study has determined the extent of prenatal toxicity and the effect of those heavy metals exposure on birth outcomes among a Myanmar population.

### *1.7 Study Objectives*

This study aimed to identify the associations between prenatal heavy metals exposure and adverse birth outcomes among pregnant women and newborns in Myanmar. This study also aimed to determine the effect of prenatal heavy metals exposure on fetal cellular damage by observing newborn leucocyte TL as a biomarker. For a better understanding of the etiologic pathway, this study also explored the associations between TL and adverse birth outcomes. The study objectives are also presented in the following schematic diagram (Figure 1). The findings of this study highlight heavy metals-induced newborn leucocyte TL alterations and its relationship with adverse birth outcomes which could be beneficial for a better understanding of the fetal origin of adulthood diseases.

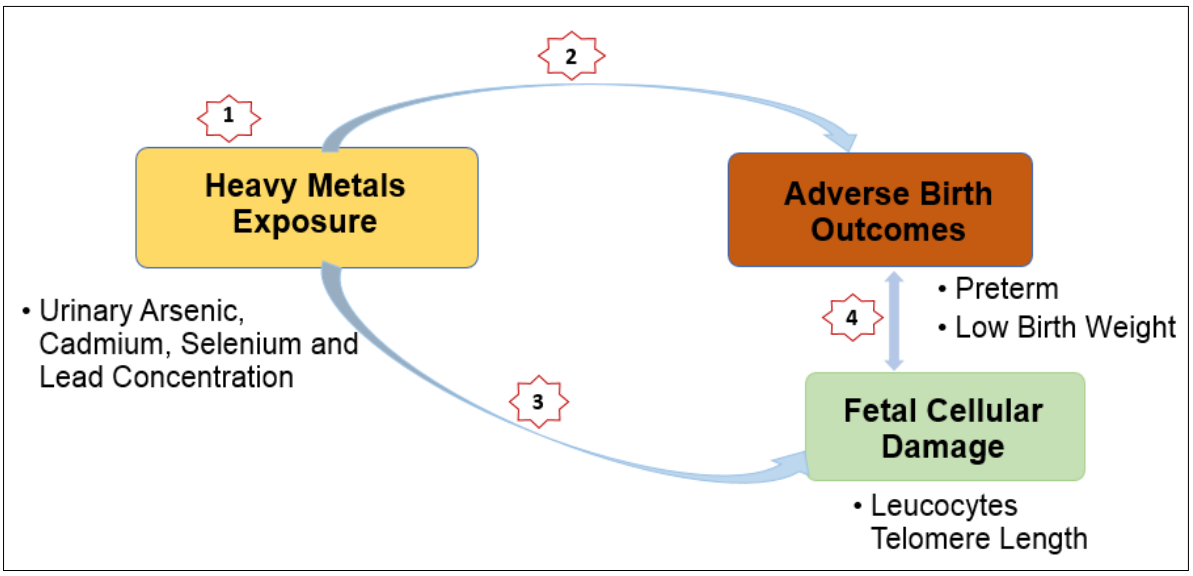


**Figure 1: Conceptual Framework**

# Chapter 2: Methods

## *2.1 Study Design and Area*

A birth-cohort study was conducted on 493 pregnant women and newborns in the Kyaungone and Kyonpyaw Districts of the Ayeyarwady Region, Myanmar (Appendix 1). Face-to-face interviews were performed using a questionnaire, and maternal spot urine samples were collected during the third trimester. Cord blood samples and birth outcomes were evaluated at delivery during the follow-up period of one to three months.

The Ayeyarwady Region is bound on the north by the Bago Region; on the east by the Yangon Region and on the south and west by the Bay of Bengal. Geographically, the region encloses an area between north latitudes 15° 40' and 18° 30' and between east latitudes 94° 15' and 96° 15', covering a total area of 35,140 $km^2$, with a total population of 6,184,829. According to Census 2014, the population density in this region was 180/$km^2$. The Ayeyarwady Region has been identified for its high concentration of arsenic in the groundwater. According to a national report, of a total tested 123,962 drinking water samples in 17 townships, approximately 29% contained an arsenic level above 10 μg/L, and about 8.2% were higher than 50 μg/L [149]. In terms of water sources, shallow or deep tube wells (76.4%) ranked first for highest contamination followed by dug wells (21.6%) and surface water (1.9%) [149]. Census 2014 reported that approximately 96% of the population relied on tube wells for their primary source of drinking water in the Ayeyarwady Region.

Three hospitals within approximately two hours of driving participated in this study. The included hospitals were township- or station-level public hospitals intended to assure accessibility of health services for the general population. All hospitals were checked for the availability of basic infrastructure such as electric supply and storage areas, during the preliminary situational survey in June, 2016.

### *2.2 Data Collection Period*

Data collection was conducted from August to December 2016.

### *2.3 Participants*

Eligible participants were pregnant women aged 18 years and above; residing in the study area for more than six months who visited to the health center for antenatal care services in the third trimester and with their newborns. Pregnant women who suffered from severe medical conditions and those who did not give consent were excluded from this study.

### *2.4 Sample Size*

Data regarding prenatal heavy metals exposure and newborn TL is not yet available. In addition, there was no information on the prenatal heavy metals exposure among the Myanmar population. Therefore, the sample size was estimated as follows.

Sample size = $\frac{z^2 * p\,(1-p) / e^2}{1 + \frac{(z^2 * p\,(1-p))}{e^2 N}}$, where z = z-score at 95% CI, p = probability, e = margin of error of 5%, and N = population size, assuming that 50% of the population was at risk since 58% of the wells in the Ayeyarwady Region had an arsenic concentration of > 10 µg/L in a previous study [147]. The estimated sample size turned out to be 384. Considering the missing value, a total of 493 participants were recruited for this study.

*2.5 Terminology and Operational Definitions*

(1) Pregnant women: Women who carry a developing fetus in their uterus, certified by medical professionals.

(2) Newborns: Newborns are just born babies who are only hours old. In this study, newborns mean both live births (born alive) regardless of birth outcomes and stillbirths (born dead).

(3) Normal pregnancy outcome: In this study, normal pregnancy outcome was defined as term delivery without any complication.

(4) Adverse birth outcomes: In this study, adverse birth outcomes included low birth weight (birth weight < 2500 g at term), preterm delivery (a live birth before 37 weeks of completed gestation), stillbirth and congenital abnormalities [153].

(5) Skilled birth attendants: Skilled birth attendants are skilled health personnel (including doctors, nurses and midwives and not including auxiliary midwives and traditional birth attendants) who have been educated and trained to be proficient in the skills needed to manage normal pregnancies, childbirth, and the

immediate postnatal period as well as in the identification, management, and referral of complications in women and newborns.

(6) Telomere length (TL): In this study, relative TL was measured by quantitative real-time polymerase chain reaction (PCR). TL was measured as the ratio of telomere repeat unit signals to a single copy gene [154].

*2.6 Data Collection*

All of the field work was conducted in collaboration with the local health centers. A material transfer agreement was obtained from the Department of Physiology, the University of Medicine 1, Yangon and the Department of Medical Research, Ministry of Health and Sports, Myanmar to bring back the biological samples to Japan for further analysis.

Before the actual data collection, advocacy and training sessions were conducted by the principal researcher at the local health centers. The training session covered the objectives of the study, and included an explanation of the questionnaire (Appendix 2) and the sampling procedure. Proper instruction (Appendix 3 and 4) was informed to all of the local health personnel before biological sample collection.

Pregnant women who met the eligible criteria were selected from a list of antenatal attendance from the district or station health centers. The district or station health centers manage all of the maternal and child health programs, including the antenatal care services for all sub-divisions of the respective district. Authorized health personnel of each sub-division then assisted the research team to trace the eligible pregnant women. Those women were approached for in-person interviews and were

asked for the drinking water and urine samples. In Myanmar, standardized antenatal care is aimed at ensuring that every pregnant woman can have access to at least four antenatal visits with quality care by the skilled birth attendants without any financial burden [155]. According to the recent Demographic and Health Survey 2015-16, approximately 78% of women receive antenatal care from skilled birth attendants in the Ayeyarwady Region [152].

During the first visit, each participant underwent a pretested face-to-face interview for about 30 to 45 minutes by the principal investigator and trained research assistants in Myanmar. Questionnaires were initially prepared in English and then translated to Myanmar. To enhance the accuracy, it was back-translated into English by an independent healthcare staff. A maternal spot urine sample was also collected at the first visit. The participants were also asked for a drinking water sample of 20 mL by the research team.

During the follow-up (after 1 to 3 months), the skilled birth attendants collected cord blood (3 - 5 mL) at the time of delivery in an ethylene-diamine-tetra-acetic acid (EDTA)-coated tube under aseptic conditions. The local skilled birth attendants performed the anthropometric measurements and noted the information regarding the birth outcomes in the delivery records after birth. It was then extracted for further analysis in this study.

All samples were collected in sterile bottles with respective seals and labels. Water samples were properly acidified with nitric acid (grade for analysis of poisonous metals, 60%, Wako, Osaka, Japan) at a ratio of 4.5 mL water with 75 μL of 60% nitric acid. All samples were firstly stored at - 20 °C at the local health centers. The frozen

samples were then transported to the Department of Human Ecology, the University of Tokyo, Japan under a cold chain and kept frozen at - 80 °C until experimental analysis.

### *2.7 Measurements*

#### *2.7.1 Assessment of Heavy Metals Exposure*

Arsenic, cadmium, selenium and lead concentrations in water and maternal urine were measured by an assured quality-controlled technique using octapole collision/reaction cell inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce ICP-MS, Agilent Technologies, Santa Clara, CA, USA). Original urine samples were diluted 20-fold with 1% nitric acid (grade for analysis of poisonous metals, 60%, Wako, Osaka, Japan) and 2% 1-butanol (grade for HPLC, 99.5% Nacalai Tesque, Kyoto, Japan), and filtered through a 0.45 μm pore membrane (Millipore, Billerica, MA, USA) connected to a disposable plastic syringe. ICP multi-elements standard solution (XVI CertiPUR, Merck, Darmstadt, Germany) was prepared by the gravimetric method to generate a calibration curve. The detection limit (DL) was calculated as three times standard deviation (SD) of the procedural blanks. The average DL values of arsenic, cadmium, lead and selenium were 0.239, 0.025, 0.843 and 0.362 μg/L, respectively for urine and 0.015, 0.013, 0.049 and 0.017 μg/L, respectively for water. Values under the DL were assumed as a half value of DL. Analytical quality was assured by the repeated analysis of the samples against the National Institute of Standards and Technology (NIST) Standard Reference Material 1643f Trace Elements in Water (NIST, Gaithersburg, MD, USA), National Institute for Environmental Studies

(NIES) Certified Reference Material No.18 Human Urine (NIES, Ibaraki, Japan) and Seronorm Trace Elements Urine (SERO AS, Billingstad, Norway).

In this study, maternal urine was used as a biomarker of heavy metals exposure. For the studies of a large sample size, less invasive and highly accessible samples are relevant as biomarkers [156]. Among this study population, ingestion is a major route of exposure, and the amount of exposure may not vary largely day-by-day. Therefore, urine samples are expected to reflect the heavy metals exposure among the population residing in the study area for considerably long period of time. Urinary arsenic level is considered as a robust biomarker of exposure, and is widely used in many epidemiological studies since arsenic in the body is mainly excreted in the urine [156]. Urinary cadmium level is also an appropriate biomarker because urinary cadmium concentration is proportional to the cadmium body burden and accumulation in the kidneys [18]. In case of lead, since ingested and inhaled lead is rapidly distributed into blood and soft tissues, plasma/blood lead concentration is commonly used as a biomarker of exposure; urinary concentration of lead is correlated with plasma/lead concentration ($r = 0.82$, $p < 0.01$) [2, 157, 158]. Generally, plasma/blood selenium level is considered as a good indicator of selenium status in humans since it reflects the nutritional status/intake of selenium more directly [31]. Although urinary selenium level is not a robust biomarker, it is also applied in many studies since ingested selenium is mainly excreted by urinary route [35, 159], and some studies also reported the significant correlations between urinary and plasma ($r = 0.52$, $p < 0.001$) selenium concentrations [160].

All urinary heavy metals concentration measurements were adjusted for creatinine. Although 24-hours urine collection is the standard method, creatinine-adjusted single spot urine had been used to estimate the heavy metals exposure [161-163]. Considering the complexity and burdens of data collection, spot urine collection has frequently used in epidemiological studies that target a large number of subjects [163]. The urinary creatinine concentration was measured by the *in-vitro* colorimetric Jaffe method using a commercial kit (LabAssay Creatinine Kit, Wako, Osaka, Japan). A linear standard curve was drawn based on five assigned doses of standard solution. Absorbance was read at 520 nm on a spectrometer (Molecular Devices SpectraMax, Sunnyvale, CA, USA), and the creatinine concentration of each 10-fold diluted urine sample was calculated accordingly.

### *2.7.2 Assessment of Birth Outcomes and Covariates*

The pretested questionnaire covered the variables regarding sociodemographic characteristics, drinking water status, anthropological measures, smoking status, pregnancy and obstetric history (Appendix 2). Sociodemographic characteristics included age, education, occupation, ethnicity and monthly income. Information regarding the household drinking water status was also assessed through the questionnaire. It included the primary source of drinking water, methods of treatment, any information about arsenic testing in the past and reported results. Delivery records included information regarding the birth such as birth weight, the baby's sex, mode of delivery, gestational age, and other biological attributes of both mothers and newborns. Gestational age at birth was calculated by the date of the last menstrual period as noted

in delivery records and the date of childbirth. Low birth weight refers to a birth weight of less than 2500 g at term [56] and preterm delivery refers to live delivery before 37 weeks of completed gestation in accordance with the definition of the International Classification of Diseases by the WHO [142]. The birth outcome variables were then dichotomized accordingly.

### *2.7.3 Measurement of Newborn Leucocyte TL*

Genomic DNA was extracted from cord blood into 200 μL of solution using the QIAamp DNA Mini and Blood Mini Kit (Qiagen K.K., Tokyo, Japan), according to the manufacturer's instructions. The quality of extracted DNA samples was assured using the μDrop plate (Thermo Fisher Scientific, Vantaa, Finland). The ratio of absorbance at 260 nm to 280 nm was accepted only between 1.7 and 2.2 to maintain the purity of the DNA samples. In this study, the relative TL was measured by quantitative real-time PCR based on the previously established protocol [111, 154]. PCR was performed on a Roche Light Cycler Nano (Roche Diagnostics K.K., Tokyo, Japan). The temperature profiles for telomere amplification were set as 40 cycles of denaturation at 95°C for 1s, and annealing /extension at 54°C for 60 s; and for 36B4G amplification as 40 cycles at 95°C for 1s, and annealing/extension at 58°C for 60 s. In addition, melting curve analysis was applied to assure the specificity of the products. Two master mixes of PCR reagents were prepared, one with telomere primers and the other with 36B4 primers. Primers used for telomere PCR are as follows:

- tel 1b [5’-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3’] at a final concentration of 100 nM
- tel 2b [5’ -GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3’] at a final concentration of 900 nM

Primers used for single-copy gene 36B4 are as follows:

- 36B4u [5’-CAGCAAGTGGGAAGGTGTAATCC-3’] at a final concentration of 300 nM
- 36B4d [5’-CCCATTCTATCATCAACGGGTACAA-3’] at a final concentration of 500 nM

Real-time qualitative PCR determines the fractional cycle number (Ct) at which the accumulating fluorescence in the well crosses a set threshold of several standard deviations that are higher than the fluorescence. A linear plot of Ct versus the amount of targeted DNA input was generated to compare the simple relative quantitation of unknowns by the standard curve, which was derived from amplification of the serial dilutions of a reference DNA sample in the same plate. The value of crossing point deviation of the unknown sample versus the standard was extracted to calculate the TL. The relative TL was calculated as the ratio of telomere repeats product to the single-copy gene (T/S ratio) for each sample in comparison with reference DNA sample. PCR was run for twice for each sample with the respective primers. Samples were made into duplicates for each run and accepted only if the SD of the Ct values was less than 1. The linearity of the standard curves was satisfactory for both the single-copy gene (mean $r^2$ = 0.98) and the telomere (mean $r^2$ = 0.94). The inter and intra-experiment coefficients of variation (CV) for the single-copy gene were 2.9% and 5.6% respectively, and those for the telomere were 8.3% and 8.9%, respectively.

*2.8 Ethical Considerations*

This study was approved by the Research Ethics Committee of the Graduate School of Medicine, the University of Tokyo (No.11186) and the Department of Medical Research, Ministry of Health, Myanmar (ERC No.009316). A material transfer agreement was obtained through the University of Medicine 1, Yangon, Myanmar. The objectives and purposes of the study were explained to all study participants before data collection. All the participants were voluntary, and the individuals had given written informed consent.

*2.9 Data Analysis*

All data were entered and rechecked using Microsoft Excel. Statistical analysis was performed using Stata 13 (StataCorp LP, Colledge Station, TX, USA). Urinary arsenic, cadmium, selenium and lead concentrations were converted to μg/g creatinine after adjusting for creatinine over the entire analysis. Since the urinary heavy metals concentration data and relative TL data were non-normally distributed, quartiles stratification and natural log-transformation were performed to enhance the normality. A descriptive analysis was conducted to present the mean, median, interquartile range (IQR), SD and percentage. Correlations between urinary heavy metals concentration were first analyzed by Spearman's correlation. The Wilcoxon rank-sum test was applied to compare the exposure levels among different birth outcomes.

Multiple logistic regression models were used to identify the associations between prenatal heavy metals exposure and adverse birth outcomes. In the models, the dependent variable was either low birth weight (0 or 1) or preterm delivery (0 or 1). The independent variables included the creatinine adjusted heavy metals concentration of the quartile stratifications. Three different models were developed using the quartile stratifications of each heavy metal concentration where model 1 was used for bivariate analysis, model 2 was used for selenium adjustment and model 3 was used for selenium and other confounder adjustments. Associations between prenatal heavy metals exposure and TL were also assessed using similar models by bivariate and multivariate linear regression. Later, both bivariate and multivariate logistic regression analyses were performed to assess whether a shorter TL was associated with the occurrence of adverse birth outcomes.

Potential confounders, such as maternal age, ethnicity, education, gestational age, birth weight, parity, the baby's sex, mode of delivery, and smoking status were included in the regression models on the basis of rational associations in previous studies [39, 46, 52, 129, 164-166]. Regarding the smoking status, the original questionnaire of this study included four responses as "smoke before pregnancy", "smoke during pregnancy", "passive exposure" and "no exposure at all". However, only 2.1% of the study population had a history of smoking (either before or during pregnancy); therefore, this section was recategorized into only two sections as "no exposure at all" and "have or ever been or passive exposure". Since gestational age is strongly correlated with both low birth weight and preterm delivery, it was excluded in the models of adverse birth

outcomes to avoid overestimation. For all analyses, the significance level was set at *p*-value of <0.05.

## Chapter 3: Results

### *3.1 Background Characteristics of Participants*

A total of 493 participants were enrolled during their first visit. Of these, only 419 participants had complete information with regard to delivery and urine samples for analysis, and 409 participants provided the cord blood (Figure 2). The background characteristics of the participants are presented in Table 1. Of the 419 participants, the mean maternal age was 28 years with an SD value of 6.6; 74.2% of participants were Bamar ethnic and 46% had completed primary school education. Most participants were either housewives (41.9%) or farmers (36.3%). Only 2% had a history of smoking and 49.2% reported passive exposure.

[Figure 2: Participation Flow Chart of the Study]

[Table 1: Background Characteristics of Participants (n = 419)]

### *3.2 Information Regarding Household Drinking Water*

Table 2 presents information regarding household drinking water among the study population. Overall, a bore well or household pumping of groundwater was the most common primary source of drinking water in this study area (89.9%). Only 18.5% of participants were aware that their water had been previously tested for arsenic. The majority of participants (84.0%) practiced some kind of water treatment method before drinking. As shown in Figure 2, the most frequent method was traditional cloth filtration

(70.2%), followed by boiling (18.9%), settling down (13.0%), using filtering equipment/machine (5.7%), chlorination (3.2%) and others (1.0%).

[Table 2: Information Regarding Household Drinking Water (n = 493)]

[Figure 3: Methods of Household Drinking Water Treatment (n = 414)]

*3.3 Heavy Metals Exposure*

Heavy metals concentration in the household drinking water is presented in Table 3. Of the total 248 drinking water samples; arsenic concentration ranged from 0.02 μg/L to 197.90 μg/L (median = 2.10 μg/L, IQR; 0.48 - 8.79); 22.6% of drinking water samples were higher than the WHO standard of 10 μg/L (Appendix 8).

Table 4 shows the creatinine-adjusted heavy metals concentrations in maternal urine. The median values of adjusted maternal urinary concentrations of arsenic, cadmium, selenium, and lead were 74.2 μg/g creatinine (IQR, 45.49 - 126.7), 0.9 μg/g creatinine (IQR, 0.5 - 1.4), 22.6 μg/g creatinine (IQR, 17.8 - 29.8), and 1.8 μg/g creatinine (IQR, 1.0 - 3.4), respectively. The correlation matrix for the concentration of heavy metals in maternal urine is presented in Table 5. A strong positive correlation was found between the metal pairs, such as arsenic-cadmium (Spearman's rho = 0.22), arsenic-selenium (Spearman's rho = 0.36) and arsenic-lead (Spearman's rho = 0.20),

regardless of creatinine adjustment. As shown in Table 6, a significant positive correlation was also found between the drinking water arsenic concentration and maternal urinary arsenic concentration (Spearman's rho = 0.30, $p < 0.001$).

[Table 3: Heavy Metals Concentration in Household Drinking Water (n = 248)]

[Table 4: Maternal Urinary Heavy Metals Concentration (n = 419)]

[Table 5: Correlation Matrix of Maternal Urinary Heavy Metals Concentration (Spearman's rho, n = 419)]

[Table 6: Correlations between Heavy Metals Concentration in Drinking Water and Maternal Urine (Spearman's rho, n = 240)]

*3.4 Maternal Health and Delivery Record Information*

Table 7 presents information regarding the pregnancy and childbirth of the participants. The mean gestational age was 38.5 weeks (SD = 1.9). Of the 419 participants, 67.5% received antenatal care for more than four times, and their first

antenatal visit was at the gestational age of 15.6 weeks (SD = 6.1). Among the newborns, 56.8% were male and 43.2% were female. The birth weight ranged from 1510 g to 6300 g, with an average value of 3171.7 g (SD = 493.0). In total, 19% were born prematurely and 6% were born as low birth weight babies.

[Table 7: Maternal and Newborn Characteristics (n = 419)]

*3.5 Prenatal Heavy Metals Exposure and Birth Outcomes*

Tables 8a, 8b and 8c show the associations between maternal urinary heavy metals concentration and dichotomous outcomes of low birth weight and preterm delivery. Bivariate analysis revealed that an increased risk of low birth weight was not significantly associated with a higher maternal urinary cadmium concentration (lowest vs highest, odds ratio (OR) = 2.49, 95% CI: 0.90, 6.78, $p = 0.078$). The association was significant (lowest vs highest, adjusted OR (AOR) = 4.79, 95% CI: 1.25, 18.37, $p = 0.022$) after adjusting for maternal age, maternal education, the baby's sex, smoking status, primigravida and antenatal visits in the multivariate logistic regression. Additional models were also performed including the gestational age and the finding was not different. Maternal education (AOR = 0.63, 95% CI: 0.41, 0.98, $p = 0.039$) and primigravida (AOR = 3.73, 95% CI: 1.15, 12.13, $p = 0.029$) were also found to be strong predictors of low birth weight. There was no significant association between maternal heavy metals concentration and preterm delivery.

[Table 8a: Associations between Prenatal Arsenic Exposure and Adverse Birth Outcomes (n = 419)]

[Table 8b: Associations between Prenatal Cadmium Exposure and Adverse Birth Outcomes (n = 419)]

[Table 8c: Associations between Prenatal Lead Exposure and Adverse Birth Outcomes (n = 419)]

*3.6 Prenatal Heavy Metals Exposure and Newborn Leucocyte TL*

The median relative TL of the study population was 0.9 (IQR, 0.5 - 1.3). For each heavy metal (arsenic, cadmium and lead), three different models were developed to examine the associations between heavy metals exposure and TL as presented in Tables 9a, 9b and 9c. Model 1 was the bivariate analysis of heavy metals concentration and TL. The linear regression model 2 was adjusted for selenium concentration to examine the mediation effect of selenium. Model 3 was developed by including potential covariates such as maternal age, education, ethnicity, smoking status, gestational age, primigravida, mode of delivery, the baby's sex and birth weight in addition to selenium concentration. As shown in Table 9a, on bivariate analysis, urinary arsenic concentration was significantly associated with a shorter TL (lowest vs highest, coefficient = - 0.17, 95%

CI: - 0.26, - 0.08, $p < 0.001$). The associations remained significant even after adjusting for confounders; newborns with higher prenatal arsenic exposure had a shorter TL (lowest vs highest, coefficient = - 0.13, 95% CI: - 0.22, - 0.03, $p = 0.002$) (Table 9a). Similarly, cadmium concentration was significantly associated with a shorter TL across quartiles (lowest vs highest, coefficient = - 0.19, 95% CI: - 0.28, - 0.08, $p < 0.001$) (Table 9b). As presented in Table 9c, there was also a negative association between lead concentration and newborn TL (lowest vs highest, coefficient = - 0.11, 95% CI: - 0.20, - 0.02, $p = 0.020$). Selenium concentration did not significantly buffer TL shortening, induced by arsenic, cadmium or lead exposure.

[Table 9a: Associations between Prenatal Arsenic Exposure and Newborn Leucocyte TL (n = 409)]

[Table 9b: Associations between Prenatal Cadmium Exposure and Newborn Leucocyte TL (n = 409)]

[Table 9c: Associations between Prenatal Lead Exposure and Newborn Leucocyte TL (n = 409)]

*3.7 Adverse Birth Outcomes and Newborn Leucocyte TL*

Bivariate analysis followed by multivariate logistic regression was performed to identify associations between adverse birth outcomes and newborn leucocyte TL. As

shown in Table 10, there was no significant association between newborn TL and any of the adverse birth outcomes, specifically, low birth weight (AOR = 0.76, 95% CI: 0.22, 2.69, $p = 0.676$) and preterm delivery (AOR = 1.34, 95% CI: 0.60, 3.03, $p = 0.476$).

[Table 10: Associations between Adverse Birth Outcomes and Newborn Leucocyte TL (n = 409)]

# Chapter 4: Discussion

## *4.1 Summary of Findings*

To the best of my knowledge, this is the first study to explore the effect of prenatal heavy metals exposure on newborn TL. Moreover, this is the first report on the prenatal heavy metals exposure and adverse birth outcomes among a Myanmar population. This study revealed that environmental cadmium exposure is comparatively high in Myanmar. Prenatal cadmium exposure had a significant association with the likelihood of a low birth weight while preterm delivery was not significantly influenced by prenatal heavy metals exposure. Prenatal arsenic, cadmium and lead exposure significantly shortened the newborn leucocyte TL. The associations were robust even after adjusting maternal age, education, ethnicity, smoking status, parity, gestational age, mode of delivery, the baby's sex and baby's weight. The underlying pathophysiology of adverse birth outcomes was not associated with TL shortening in this study.

## *4.2 Heavy Metals Exposure*

This study firstly examined the extent of heavy metals contamination among the Myanmar population. The arsenic concentration of drinking water in this study (22.6%) was lower than the previous national report which reported that the arsenic concentration in 29.18% of samples was higher than the WHO standards of 10 μg/L [149]. This is probably because previous reports directly examined arsenic contamination in the source of drinking water while this study assessed the household drinking water where treatment and practices before drinking and handling water may

have influenced the contamination level. Since there is limited information on the concentrations of other heavy metals in Myanmar, the findings of this study support the baseline evidence of cadmium, selenium and lead concentrations in the household drinking water.

While assessing heavy metals exposure using biological samples, the urinary arsenic concentration among the Myanmar population (74.22 μg/g creatinine) was much lower than other exposed populations of Nepal (mean = 196 μg/g creatinine) and India (mean = 290 μg/L) [101, 111]. The exposure level of participants examined in this study was also lower than the concentration of an unexposed Argentinean population (median = 230 μg/L), and Bangladesh population (mean = 154 μg/L) [54, 103]. In the case of cadmium, the urinary cadmium concentration in this study (geometric mean (GM) = 0.86 μg/g creatinine) was comparatively higher than previous findings of non-industrialized polluted areas of the U.S. (mean = 0.46 μg/g creatinine) and suspected contaminated areas of South Africa (GM = 0.27 μg/g creatinine) [167, 168]. The exposure level was also higher on comparison with high rice-consuming countries, such as Bangladesh (median = 0.63 μg/g creatinine), Nepal (GM = 0.33 μg/g creatinine), and China (GM = 0.55 μg/g creatinine) [50, 52, 111]. The median selenium concentration of this study was lower than 30 μg/g creatinine which is similar to the concentration of the normal reference range [169]. The urinary lead concentration in the present study was also found to be in a similar range to those of previous reports from Japan and Korea [47, 170].

This study also found significant correlations between metals pairs in the maternal urine regardless of creatinine adjustment. Even though the possibility of over-

adjustment by creatinine could not be ruled out, this is in consistence with the previous studies which showed the correlations between urinary cadmium-selenium pair (r = 0.16, $p = 0.02$), cadmium-lead pair (r = 0.49, $p = 0.00$), and arsenic-selenium pair (r = 0.68, $p < 0.01$) [46, 171]. The results are conceivable since accumulation of one metal in body could contribute the potential excretion of others. For example, selenium intake can influence arsenic methylation, metabolism and urinary excretion [171]. Therefore, the correlations are expected because of the coexistence of metals in the body.

In this study, there was a strong positive correlation between arsenic concertation in drinking water and maternal urine (Spearman's rho = 0.30, $p < 0.001$). The finding of this study suggested that the source of arsenic exposure is probably from contaminated drinking water through ingestion. However, regarding cadmium, lead and selenium, there was no positive correlation between the drinking water and maternal urine, suggesting the importance of tracing the other sources of contamination. Future interventions should focus to increase the public awareness on arsenic removal and treatment to improve safer water supply.

This is the first study to detect a comparatively higher concentration of cadmium among a Myanmar population. Although smoking being a major source of cadmium exposure, there was no significant association between smoking status and urinary cadmium concentration in this study. Therefore, these results suggest that the main source of cadmium exposure in Myanmar is the diet (either water or food). In a previous study comparing the cadmium content in different food items, the GM for cadmium in rice (50 ng/g) was significantly higher than that of wheat-derived foods such as bread (16 ng/g), flour (19.3 ng/g) and noodles (4 ng/g) [172]. Previous studies also revealed

that cadmium from the soil was absorbed and retained in rice to a great extent [173], and that cadmium in rice exclusively correlates with the cadmium body burden [174]. In Myanmar, rice is the staple food and other rice-derived foods are also major components of daily meals. Therefore, it is important to trace the potential sources of cadmium contamination while tackling related health concerns in Myanmar.

### *4.3 Prenatal Heavy Metals Exposure and Birth Outcomes*

This study identified an association between maternal urinary cadmium concentration and an increased likelihood of a low birth weight. Low birth weight is considered a significant public health concern since it is highly associated with neonatal mortality and disease risk in adulthood [175]. Many studies have discussed the effects of arsenic, cadmium and lead exposure on several birth outcomes [39, 43, 46-49, 176, 177]. Among them, cadmium was found to have the most distinct effects on anthropometric measures of newborns. For example, a study conducted among the Saudi Arabian population reported that the cadmium concentration in umbilical cord blood was inversely correlated with crown-heel length, birth weight, Apgar 5-minute scores and small for gestational age whereas it was not associated with lead or mercury concentration in the same population [43]. This finding is also consistent with previous studies where prenatal cadmium exposure led to decreased growth of the fetus *in utero*, leading to decrease in birth weight among the exposed population of similar concentrations in Bangladesh (median = 0.63 μg/L), Saudi Arabia (mean = 0.99 μg/L) and Japan (GM = 0.77 μg/L) [43, 47, 177]. This effect remained significant at a lower concentration (GM = 0.25 μg/L ) among a South Africa coastal population, suggesting

that even a lower concentration of cadmium exposure may trigger alterations in fetal growth [168].

The underlying mechanisms of cadmium induced low birth weight have been postulated in many previous studies. Cadmium may interfere with zinc transfer to the fetus resulting in intrauterine growth retardation [178]. Cadmium may also be involved in fetoplacental hormonal alterations such as in the production of placental progesterone, thyroid stimulating hormone and placental leptin synthesis which have been linked to impaired fetal growth [24, 25, 179]. Moreover, experimental studies have provided supportive evidence that cadmium may impair placental circulation, inhibiting the transport of nutrients from the mother to the fetus [180, 181]. In contrast with previous studies, no association was found between maternal arsenic and lead exposure and low birth weight in this study [49, 176, 182]. This could be explained by the comparatively low concentrations of urinary arsenic and lead among our study population since exposure dose and timing play a critical role in intrauterine fetal growth [183].

This study also aimed to identify associations between prenatal heavy metals exposure and preterm delivery. However, in this study, prenatal heavy metals exposure was not significantly associated with preterm delivery. This finding is consistent with a previous report in which the arsenic concentration in drinking water was not significantly associated with an increased risk of preterm delivery in Taiwan [51]. In the case of cadmium, the result is contradictory to previous findings which showed that the preterm birth rate was higher with an increased urinary cadmium concentration in China and the incidence of preterm delivery among the higher urinary cadmium concentration group (≥ 2 nmol/mmol creatinine) was higher than among those with a lower cadmium

concentration in Japan [52, 184]. Regarding lead exposure, the result was in line with a previous study, conducted among Swedish and Polish women, which revealed that the lead concentration in the myometrium and placenta was not significantly elevated in preterm delivery compared to term delivery [185] while other studies in China reported that a higher maternal blood lead level (≥ 10 μg/dL) doubled the risk of preterm delivery [186]. The inconsistencies in results may be explained by differences in exposure levels and sensitivity of the population which may vary in response to exposure. Moreover, gestational age was typically estimated to determine preterm delivery based on the date of last menstrual period and/or ultrasound data [142]. The variability in gestational age by regions and assessment protocols may also explain the inconsistencies.

*4.4 Prenatal Heavy Metals Exposure and Newborn Leucocyte TL*

In the current study, prenatal arsenic exposure was negatively associated with newborn TL. A handful of experimental and epidemiological studies have assessed the associations between arsenic exposure and TL. However, the findings are limited and disputable. For example, there are few contradictory findings that showed a positive association between arsenic exposure and TL. Specifically, a study conducted in India revealed that arsenic-exposed individuals with skin lesions had a significantly longer TL than control subjects [101]. Another study in Bangladesh also reported that a longer TL was found among the high exposure group compared to the low exposure group [100]. Meanwhile, some studies proposed that arsenic exposure had shortened TL and decreased cell survival in animal models and *in vitro* studies [79, 98]. Consistent with

these results, a recent study among Italian young adults also reported a negative significant effect of arsenic exposure on leucocyte TL [102].

Contextually, the observation of the current study is plausible when taking into consideration the mechanisms of arsenic toxicity, such as the production of ROS, and alterations in the DNA repair system which, in turn, could result in telomere shortening [65, 79]. Telomeric DNA is more vulnerable to attack by ROS because of its guanine-rich structures [65]. Telomeric DNA repair function can be affected directly by arsenic-induced ROS through DNA strands breaking or by activation of genes involved in the repair of telomeric DNA [65, 100]. In addition, telomerase activity can be interfered with the oxidative DNA damage through subsiding the binding of shelterin complex proteins such as TRF1 and TRF2, leading to a deterioration in telomere maintenance [187]. The efficacy of arsenic metabolism may also play a role since it can modify the effects on telomeres; slower arsenic metabolism could result in a higher risk of arsenic-induced TL alterations and carcinogenicity [103]. The shortened TL observed in this study could be due to the direct and/or indirect damage of TL by arsenic through the above-mentioned pathways. In contrast to previous studies among highly-exposed populations with clinical manifestations [100, 101, 103], the exposure level in this study was comparatively lower. Moreover, this study examined newborns' TL to diminish the combined carcinogenic effects developed by other environmental factors, and the duration of exposure was relatively short in our study population. Therefore, the inconsistent results are coherent since telomere lengthening could be expected from long-term exposure of arsenic and the carcinogenic mode of arsenic may also be

involved in telomere lengthening of pre-malignant cells to increase the lifespan [100, 101].

Upon quartile stratification of cadmium exposure, this study found a significant negative trend across quartiles although the linear association was not significant. Our finding is consistent with the previous study among eight years old children in Poland which found no linear association between cadmium exposure and TL [78]. Our results are also consistent with previous findings, which showed that cadmium exposure (highest quartile vs lowest quartile) was inversely associated with TL (β = - 5.54; 95% CI: - 0.42, - 0.47 for blood and β = - 4.50; 95% CI: - 8.79, - 0.20 for urine) among the U.S adults [110]; and another study among Nepalese adolescents that reported a significant linear downward trend between cadmium quintiles and salivary TL ($P_{trend}$ = 0.01) [111]. However, our results contradict a study in China, which reported that placental cadmium concentration was linearly associated with placental telomere shortening (r = - 0.138, $p$ = 0.013) [23].

The effect of cadmium on TL has been biologically postulated in many previous studies. Cadmium induces oxidative stress and inflammatory chemicals that can potentially accelerate TL shortening [84, 85]. Moreover, cadmium is also an established mutagenic metal, involved in the DNA repair system either through ROS induction or through enzymatic reaction due to its divalent ion action in replacing zinc ions [114, 115]. The non-linear association revealed in this study could be explained by the dose of cadmium exposure passing the placental barrier during pregnancy and the differing study population. Although both inorganic arsenic and arsenic metabolites can freely pass through the placental barrier [16], cadmium rather accumulates in the placental

tissues; thus, the placental cadmium level was highly correlated with the maternal blood cadmium level [38, 42] whereas the maternal blood cadmium level did not correlate with cord blood cadmium level [38, 188]. Taken together, it is possible that newborn TL was unviolated due to the limited transfer of cadmium from the mother to the fetus except at higher concentrations. Therefore, the non-linear association and the negative quartile trend between cadmium exposure and newborn TL are conceivable.

In this study, prenatal lead exposure was also negatively associated with newborn TL. In accordance, a study in Poland reported that the blood lead level was inversely associated with leucocyte TL among both children ($r = -0.25$, $p = 0.013$) and adults ($\beta = -0.004$; $p = 0.006$) [78, 116]. Another study among Chinese battery workers also found an inverse association between the blood lead level and peripheral white blood TL ($r = -0.70$, $p < 0.0001$) [117]. In contrast, our finding is contradictory to previous results where lead exposure was not associated with placental TL among a Chinese population [23] and leucocyte TL among a U.S. population [110].

Biologically, the effect of lead exposure on TL was recognizable since lead has been proven to promote ROS production; by inducing oxidative stress [1, 121]. The divalent ionic action of lead could also interfere with the uptake of certain elements such as zinc, calcium and magnesium which may ultimately affect DNA repair enzymes [26, 121]. Meanwhile, a recent study that examined blood heavy metals concentration among pregnant Korean women and their children up to five-years old revealed that lead concentration was lowest in the cord blood and highest in 24-36 month-old children, suggesting that children are at a higher risk of lead contamination from more hand-to-mouth activities at those ages [189]. The effect of lead on TL may be expressed

even at a low concentration and cumulative exposure should be considered since it is possible that lead exposure may affect TL even after birth in early childhood.

This study found no buffering effect of selenium on newborn TL shortening induced by arsenic, cadmium or lead. So far, no previous epidemiological study has discussed the effect of selenium on TL among a human population. Some experimental studies have reported that selenium prevents the shortening of TL on yeast cells due to its antioxidant property and it was also shown to exhibit an antimutagenic effect by inhibiting telomerase activity in cancer cells [190, 191]. However, the present study found no mediation effect of selenium which could probably be explained by the interaction between heavy metals that may overcome the effect of selenium on newborn TL.

The findings of the present study underline the importance of heavy metals exposure during prenatal life which results in TL shortening. In this study, newborns of the highest exposure group had 11% to 19% shorter TL than the lowest exposure group. This illustrates that newborns of highly exposed mothers are biologically older than newborns of unexposed mothers in terms of TL. This variation at birth may diminish the buffering capacity of TL to postnatal influence which may increase the risk of adulthood diseases.

*4.5 Adverse Birth Outcomes and Newborn Leucocyte TL*

This study found that newborn leucocyte TL was not significantly associated with either low birth weight or preterm delivery. Our finding is consistent with a

previous report of three cohort studies in Finland, which showed that leucocyte TL did not correlate with birth weight, birth height or gestational age at birth in any of the cohorts [192]. Our result is also in line with a previous prospective cohort study in the U.K., which reported that small for gestational age babies and their mothers had no significant shorter TL than term babies [193]. Together with the previous findings, our results support the idea that the pathophysiology behind low birth weight and preterm delivery was not correlated with newborn telomere homeostasis.

In contrast, there are also differing results among previous studies. For example, a previous study on TL among preterm (< 37 gestational weeks) and full-term (> 37 gestational weeks) babies found a significant decrease in TL between 27 and 32 weeks of gestation while no association was observed between 33 and 42 weeks of gestation [194]. These results are in concordance with the explanation that the telomerase activity of placental tissues is highest during the first trimester and decreases over time during pregnancy [126]. Therefore, the conflicting results are understandable since our study recruited pregnant women in their third trimester (mean gestational age = 38.5 weeks) to minimize the physiological confounding effect of gestational age on TL. In another study, placental TL was significantly lower among intrauterine growth restriction babies compared to normal uncomplicated control babies [132]. This inconsistency could be explained by the different nature of samples where the present study measured newborn leucocyte TL. Placental TL is considered an important factor in the physiology of placental development [125, 131, 132]. Across the gestation period, placental cells replicate rapidly to fulfil the increasing demands of fetal growth [125]. As an adaptive response, placental TL shortening indicates placental aging during pregnancy [131, 132].

Therefore, it is possible that the placental TL may be more responsible for the effects of adverse birth outcomes while maintaining fetal homeostasis than fetal TL.

### *4.6 Strengths and Limitations*

This study drew on many important strengths. The study design was a birth-cohort prospective design and included a broad information on potential confounders. The study was conducted at three public general hospitals to minimize the selection bias. Birth outcomes data were extracted from the medical records of township- and district-level hospitals where well-trained health professionals measured and recorded the birth information. In addition, before the actual data collection, advocacy and training sessions were given to the local health professionals in advance to make them aware of the significance of obtaining accurate information. The concentrations of heavy metals were measured using a sensitive, robust and well-validated method (ICP-MS), and was assured with certified reference materials. Multiple important heavy metals were examined rather than focusing on only one heavy metal. TL was measured from a highly accessible source of DNA, blood leucocytes since leucocyte TL is largely heritable and the attrition may be influenced by environmental factors [195, 196]. Moreover, this is the first study to determine whether prenatal heavy metals exposure affects newborns TL. This is also the first report on the extent of prenatal heavy metals exposure and its associations with adverse birth outcomes among a Myanmar population.

Some limitations should also be addressed in this study. The present study failed to control the genetic factors and prenatal exposure to other toxic chemicals which could collectively affect the outcomes. Nutritional intake was not fully considered in this study, although it is an important predictor of low birth weight [197]. Although the antenatal attendance is more than 78% in this study area [152], there is the possibility of missing eligible participants. Information on birth outcomes was extracted from the hospitals' medical records, which may differ according to measurement protocols. Regarding TL, this study raised some issues to be focused on in future research. Maternal TL should be measured to investigate the mode of inheritance from mothers to newborns. It would also be worthwhile to perform a longer prospective study with repeated measurements of TL and the assessment of health outcomes later in life.

## Chapter 5: Conclusions and Perspectives

The present study provides baseline information concerning environmental heavy metals exposure among a Myanmar population. This study revealed that Myanmar mothers are comparatively highly exposed to cadmium which should be considered a public health threat. Prenatal exposure to cadmium was positively associated with low birth weight in Myanmar. Furthermore, this is the first study to reveal the impact of prenatal heavy metals exposure on newborn TL. This study identified that a strong and independent inverse association between prenatal arsenic, cadmium and lead exposure and newborn TL. However, the risk of adverse birth outcomes was not associated with newborn leucocytes TL in this study.

Based on existing research, the TL at birth predicts the TL later in life, and the findings of this study revealed that *in utero* heavy metals exposure could predict TL at birth. Therefore, future public health measures should integrate interventions to reduce heavy metals contamination with special emphasis on pregnant women. Meanwhile, compelling prospective studies are warranted to predict the early life developmental effects and ongoing health risks among individuals with a shorter TL. Considering TL as a biomarker, future insights should incorporate the dose-response effect of environmental heavy metals exposure risks to foresee preceding and/or present diseases which could provide further important insights into the mechanisms of disease processes.

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**Table 1: Background Characteristics of Participants (n = 419)**

| Characteristics | n | % | Mean | SD |
|---|---|---|---|---|
| **Age (years)** | 419 | | 27.9 | 6.6 |
| **Religion** | | | | |
| Buddhist | 376 | 89.7 | | |
| Christian | 41 | 9.8 | | |
| Others | 2 | 0.5 | | |
| **Ethnicity** | | | | |
| Bamar | 311 | 74.2 | | |
| National races† | 106 | 25.3 | | |
| Others | 2 | 0.5 | | |
| **Education** | | | | |
| Illiterate | 7 | 1.7 | | |
| Read and write | 66 | 15.8 | | |
| Primary school completed | 193 | 46.1 | | |
| Middle school completed | 81 | 19.3 | | |
| High school completed | 44 | 10.5 | | |
| Graduate and above | 28 | 6.9 | | |
| **Occupation** | | | | |
| Unemployed or housewives | 176 | 41.9 | | |
| Farmers | 152 | 36.3 | | |
| Private sectors | 8 | 1.9 | | |
| Government officers | 14 | 3.3 | | |
| Own business | 32 | 7.6 | | |
| Others | 37 | 8.8 | | |
| **Monthly household income # (USD)** | 296 | | 124.2 | 55.7 |
| **Hospitals** | | | | |
| Kyaungone | 153 | 36.5 | | |
| Kyonpyaw | 139 | 33.2 | | |
| Ahtaung | 127 | 30.3 | | |
| **Smoking status** | | | | |
| Not at all | 210 | 50.1 | | |
| Have or ever been or passively exposed | 209 | 49.9 | | |

†National races include Kachin, Kayar, Kayin, Chin, Mon, Yakhine and Shan.

# 1 USD = 1224 MMK as of Sep, 2016.

**Table 2: Information Regarding Household Drinking Water (n= 493)**

| Characteristics | Number | Percentage |
|---|---|---|
| **Primary source of drinking water** | | |
| Bore well/Hand pump/ Motor pump | 419 | 89.9 |
| Community well | 14 | 2.8 |
| River/ Lake water/Rain water | 6 | 1.2 |
| Household water supply (Municipal piped) | 10 | 2.0 |
| Others | 44 | 8.5 |
| **Duration of primary water source** | | |
| Less than 2 years | 125 | 25.3 |
| 2 – 20 years | 253 | 51.3 |
| More than 20 years | 85 | 17.2 |
| Don't know | 30 | 6.1 |
| **Any treatment before drinking** | | |
| No | 79 | 16.0 |
| Yes | 414 | 84.0 |
| **If yes, how often** | | |
| Always | 390 | 94.2 |
| Usually | 14 | 3.4 |
| Sometimes | 10 | 2.4 |
| Few | 0 | 0 |
| **Water testing** | | |
| Within 1 month | 20 | 4.1 |
| Within 1 – 3 months | 45 | 9.9 |
| Within 3 – 6 months | 73 | 14.8 |
| More than 6 months | 144 | 29.2 |
| Never | 199 | 40.4 |
| Missing | 8 | 1.6 |
| **Ever been tested for arsenic** | | |
| No | 245 | 49.7 |
| Yes | 91 | 18.5 |
| Don't know | 157 | 31.8 |
| **If yes, results** | | |
| Above standard (>= 50 μg/L) | 12 | 13.2 |
| Below standard (< 50 μg/L) | 59 | 64.8 |
| Don't know | 20 | 22 |

**Table 3: Heavy Metals Concentration in Household Drinking Water (n = 248)**

| Concentration (µg/L) | Median | IQR | Detection Limit |
|---|---|---|---|
| Arsenic | 2.10 | (0.48 – 8.79) | 0.015 |
| Cadmium | 0.01 | (0.01 – 0.01) | 0.013 |
| Selenium | 0.01 | (0.01 – 0.02) | 0.017 |
| Lead | 0.02 | (0.02 – 0.02) | 0.049 |

**Table 4: Maternal Urinary Heavy Metals Concentration (n = 419)**

| Concentration | Median | IQR | Detection Limit |
|---|---|---|---|
| **Unadjusted for Creatinine (µg/L)** | | | |
| Arsenic | 55.18 | (30.54 – 94.56) | 0.239 |
| Cadmium | 0.62 | (0.31 – 1.08) | 0.002 |
| Selenium | 16.40 | (10.19 – 25.6) | 0.361 |
| Lead | 1.33 | (0.42 – 2.89) | 0.843 |
| **Adjusted for Creatinine (µg/g creatinine)** | | | |
| Arsenic | 74.22 | (45.48 – 126.67) | |
| Cadmium | 0.86 | (0.50 – 1.40) | |
| Selenium | 22.57 | (17.79 – 29.78) | |
| Lead | 1.80 | (1.04 – 3.43) | |

**Table 5: Correlation Matrix of Maternal Urinary Heavy Metals Concentration (Spearman's rho, n=419)**

| | Arsenic | Cadmium | Selenium | Lead |
|---|---|---|---|---|
| **Unadjusted for Creatinine** | | | | |
| Arsenic | 1.00 | | | |
| Cadmium | 0.41* | 1.00 | | |
| Selenium | 0.60* | 0.64* | 1.00 | |
| Lead | 0.44* | 0.51* | 0.62* | 1.00 |
| **Adjusted for Creatinine** | | | | |
| Arsenic | 1.00 | | | |
| Cadmium | 0.22* | 1.00 | | |
| Selenium | 0.36* | 0.41* | 1.00 | |
| Lead | 0.20* | 0.27* | 0.26* | 1.00 |

* $p < 0.001$

**Table 6: Correlations between Heavy Metals Concentration in Drinking Water and Maternal Urine (Spearman's rho, n = 240)**

| Variable | Spearman's rho | *p* - value |
|---|---|---|
| Water arsenic – urine arsenic | 0.30 | < 0.001 |
| Water cadmium – urine cadmium | - 0.14 | 0.03 |
| Water lead – urine lead | - 0.02 | 0.08 |
| Water selenium – urine selenium | - 0.09 | 0.69 |

**Table 7: Maternal and Newborn Characteristics (n = 419)**

| Characteristics | n | % | Mean | SD |
|---|---|---|---|---|
| **Gestational age (weeks)** | 419 | | 38.5 | 1.9 |
| **Primigravida** | | | | |
| No | 181 | 43.2 | | |
| Yes | 238 | 56.8 | | |
| **Antenatal visits** | | | | |
| Less than four times | 136 | 32.5 | | |
| four or more than four times | 283 | 67.5 | | |
| **Gestational week of first antenatal visit** | 419 | | 15.6 | 6.1 |
| **Mode of delivery** | | | | |
| Normal spontaneous delivery | 188 | 44.9 | | |
| Assisted delivery [γ] | 7 | 1.7 | | |
| Cesarean delivery | 224 | 53.5 | | |
| **Baby's sex** | | | | |
| Male | 238 | 56.8 | | |
| Female | 181 | 43.2 | | |
| **Birth weight (g)** | 419 | | 3171.7 | 493.0 |
| **Birth outcomes** | | | | |
| Normal alive | 329 | 78.5 | | |
| Stillbirth | 2 | 0.5 | | |
| Preterm [θ] | 80 | 19.1 | | |
| Congenital abnormality | 2 | 0.5 | | |
| Low birth weight † | 26 | 6.2 | | |
| **Relative leucocyte TL (T/S ratio)** | 409 | | 0.9 [a] | (0.5 – 1.3) [b] |

[γ] Assisted delivery includes vacuum or forceps deliveries.

[θ] Any delivery before 37 weeks of gestation regardless of birth weight.

† Birth weight < 2500 g regardless of gestational age at birth.

TL: Telomere length. [a] Median. [b] IQR.

**Table 8a: Associations between Prenatal Arsenic Exposure and Adverse Birth Outcomes (n= 419)**

| Characteristics | Low Birth Weight † | | | Preterm ᵞ | | |
|---|---|---|---|---|---|---|
| | Model 1 [a] OR (95% CI) | Model 2 [b] AOR (95% CI) | Model 3 [c] AOR (95% CI) | Model 1 [a] OR (95% CI) | Model 2 [b] AOR (95% CI) | Model 3 [c] AOR (95% CI) |
| **Arsenic concentration** ᶿ | | | | | | |
| Quartile 1 (< 45.5) | ref | ref | ref | ref | ref | ref |
| Quartile 2 (45.5 – 74.2) | 1.53 (0.47, 4.98) | 1.55 (0.47, 5.13) | 1.23 (0.36, 4.25) | 0.80 (0.40, 1.59) | 0.79 (0.39, 1.59) | 0.80 (0.38, 1.66) |
| Quartile 3 (74.2 – 126.7) | 1.71 (0.54, 5.41) | 1.78 (0.54, 5.83) | 1.75 (0.51, 6.04) | 0.77 (0.39, 1.53) | 0.79 (0.39, 1.59) | 0.89 (0.43, 1.87) |
| Quartile 4 (> 126.7) | 1.40 (0.48, 4.73) | 1.41 (0.39, 5.08) | 1.13 (0.29, 4.35) | 1.06 (0.54, 2.06) | 1.15 (0.57, 2.32) | 1.28 (0.61, 2.70) |
| **Selenium concentration** ᶿ | | | | | | |
| Quartile 1 (< 17.8) | | ref | ref | | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.71 (0.23, 2.14) | 0.65 (0.20, 2.03) | | 1.84 (0.91, 3.71) | 1.55 (0.76, 3.20) |
| Quartile 3 (22.6 – 29.8) | | 0.44 (0.13, 1.55) | 0.43 (0.11, 1.60) | | 1.65 (0.81, 3.36) | 1.39 (0.66, 3.20) |
| Quartile 4 (> 29.8) | | 0.91 (0.31, 2.66) | 0.92 (0.30, 2.91) | | 0.94 (0.42, 2.09) | 0.76 (0.33, 1.74) |
| **Maternal age** (years) | | | 1.05 (0.98, 1.13) | | | 1.03 (0.98, 1.07) |
| **Maternal education** | | | 0.63 (0.40, 0.97) * | | | 0.87 (0.69, 1.11) |
| **Primigravida** (ref: non primigravida) | | | 4.29 (1.36, 13.55) * | | | 1.75 (0.90, 3.41) |
| **Antenatal visits ≥ 4 times** (ref: < 4 times) | | | 0.59 (0.25, 1.39) | | | 1.33 (0.75, 2.08) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | 0.63 (0.27, 1.48) | | | 0.42 (0.25, 0.70) ** |
| **Baby's sex** (ref: male) | | | 1.61 (0.70, 3.68) | | | 1.25 (0.75, 2.08) |
| **Smoking Status** (ref: no exposure) | | | 0.97 (0.42, 2.22) | | | 1.55 (0.92, 2.61) |

*$p < 0.05$. ** $p < 0.01$.

ᶿ Adjusted for creatinine (μg/g creatinine); † Birth weight < 2500 g regardless of gestational age at birth; ᵞ Any delivery before 37 weeks of gestation regardless of birth weight.

[a] Bivariate logistic regression; [b] Multivariate logistic regression adjusted for selenium concentration; [c] Multivariate logistic regression adjusted for maternal age, maternal education, parity, baby's sex, mode of delivery, smoking status and selenium concentration.

**Table 8b: Associations between Prenatal Cadmium Exposure and Adverse Birth Outcomes (n= 419)**

| Characteristics | Low Birth Weight † | | | Preterm ᵞ | | |
|---|---|---|---|---|---|---|
| | Model 1 [a] | Model 2 [b] | Model 3 [c] | Model 1 [a] | Model 2 [b] | Model 3 [c] |
| | OR (95% CI) | AOR (95% CI) | AOR (95% CI) | OR (95% CI) | AOR (95% CI) | AOR (95% CI) |
| **Cadmium concentration** [θ] | | | | | | |
| Quartile 1 (< 0.5) | ref | Ref | ref | ref | ref | ref |
| Quartile 2 (0.5 – 0.9) | 0.34 (0.07, 1.71) | 0.34 (0.07, 1.73) | 0.36 (0.07, 1.91) | 1.42 (0.72, 2.78) | 1.49 (0.75, 2.94) | 1.41 (0.69, 2.87) |
| Quartile 3 (0.9 – 1.4) | 0.91 (0.27, 3.09) | 1.12 (0.32, 3.91) | 1.16 (0.31, 4.40) | 1.20 (0.60, 2.40) | 1.16 (0.60, 2.37) | 1.09 (0.51, 2.32) |
| Quartile 4 (> 1.4) | 2.47 (0.90, 6.78) | 3.38 (1.10, 10.43) * | 4.79 (1.25, 18.37) * | 0.93 (0.46, 1.92) | 0.98 (0.57, 2.11) | 0.86 (0.36, 2.05) |
| **Selenium concentration** [θ] | | | | | | |
| Quartile 1 (< 17.8) | | Ref | ref | | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.65 (0.21, 2.01) | 0.53 (0.16, 1.72) | | 1.80 (0.89, 3.63) | 1.53 (0.74, 3.16) |
| Quartile 3 (22.6 – 29.8) | | 0.29 (0.08, 1.06) | 0.26 (0.64, 1.02) | | 1.75 (0.85, 3.61) | 1.53 (0.72, 3.25) |
| Quartile 4 (> 29.8) | | 0.52 (0.16, 1.66) | 0.43 (0.12, 1.49) | | 1.01 (0.45, 2.25) | 0.88 (0.38, 2.03) |
| **Maternal age** (years) | | | 0.99 (0.92, 1.08) | | | 1.03 (0.98, 1.08) |
| **Maternal education** | | | 0.63 (0.41, 0.98) * | | | 0.88 (0.69, 1.12) |
| **Primigravida** (ref: non primigravida) | | | 3.73 (1.15, 12.13) * | | | 1.79 (0.92, 3.46) |
| **Antenatal visits ≥ 4 times** (ref: < 4 times) | | | 0.54 (0.23, 1.28) | | | 1.29 (0.73, 2.28) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | 0.58 (0.24, 1.39) | | | 0.42 (0.25, 0.70) ** |
| **Baby's sex** (ref: male) | | | 1.52 (0.64, 3.58) | | | 1.26 (0.75, 2.11) |
| **Smoking status** (ref: no exposure) | | | 0.87 (0.37, 2.01) | | | 1.53 (0.91, 2.56) |

*$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

[θ] Adjusted for creatinine (μg/g creatinine); † Birth weight < 2500 g regardless of gestational age at birth; [ᵞ] Any delivery before 37 weeks of gestation regardless of birth weight.

[a] Bivariate logistic regression; [b] Multivariate logistic regression adjusted for selenium concentration; [c] Multivariate logistic regression adjusted for maternal age, maternal education, parity, baby's sex, mode of delivery, smoking status and selenium concentration.

**Table 8c: Associations between Prenatal Lead Exposure and Adverse Birth Outcomes (n= 419)**

| Characteristics | Low Birth Weight † | | | Preterm ᵞ | | |
|---|---|---|---|---|---|---|
| | Model 1 [a] OR (95% CI) | Model 2 [b] AOR (95% CI) | Model 3 [c] AOR (95% CI) | Model 1 [a] OR (95% CI) | Model 2 [b] AOR (95% CI) | Model 3 [c] AOR (95% CI) |
| **Lead concentration** [θ] | | | | | | |
| Quartile 1 (< 1.0) | ref | ref | ref | Ref | ref | ref |
| Quartile 2 (1.0 – 1.8) | 0.86 (0.32, 2.33) | 0.82 (0.30, 2.24) | 1.00 (0.34, 2.90) | 1.04 (0.52, 2.05) | 1.10 (0.55, 2.18) | 1.22 (0.60, 2.49) |
| Quartile 3 (1.8 – 3.4) | 0.57 (0.18, 1.75) | 0.57 (0.18, 1.78) | 0.56 (0.18, 1.87) | 0.94 (0.47, 1.91) | 0.94 (0.46, 1.91) | 0.98 (0.47, 2.05) |
| Quartile 4 (> 3.4) | 0.44 (0.13, 1.49) | 0.43 (0.12, 1.51) | 0.43 (0.12, 1.58) | 1.13 (0.70, 2.23) | 1.19 (0.58, 2.41) | 1.22 (0.58, 2.54) |
| **Selenium concentration** [θ] | | | | | | |
| Quartile 1 (< 17.8) | | ref | ref | Ref | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.76 (0.25, 2.29) | 0.71 (0.22, 2.23) | | 1.80 (0.89, 3.61) | 1.52 (0.74, 3.12) |
| Quartile 3 (22.6 – 29.8) | | 0.56 (0.16, 1.94) | 0.57 (0.15, 2.16) | | 1.61 (0.79, 3.29) | 1.40 (0.66, 2.96) |
| Quartile 4 (> 29.8) | | 1.21 (0.43, 3.44) | 1.19 (0.39, 3.64) | | 0.93 (0.43, 2.02) | 0.78 (0.35, 1.73) |
| **Maternal age** (years) | | | 1.04 (0.97, 1.12) | | | 1.02 (0.98, 1.07) |
| **Maternal education** | | | 0.63 (0.41, 0.98) * | | | 0.86 (0.68, 1.10) |
| **Primigravida** (ref: non primigravida) | | | 4.06 (1.31, 12.52) * | | | 1.71 (0.89, 3.29) |
| **Antenatal visits ≥ 4 times** (ref: < 4 times) | | | 0.53 (0.22, 1.26) | | | 1.30 (0.73, 2.29) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | 0.60 (0.25, 1.42) | | | 0.41 (0.25, 0.70) ** |
| **Baby's sex** (ref: male) | | | 1.61 (0.70, 3.69) | | | 1.25 (0.75, 2.08) |
| **Smoking status** (ref: no exposure) | | | 0.90 (0.39, 2.06) | | | 1.53 (0.91, 2.56) |

*$p < 0.05$. ** $p < 0.01$.

[θ] Adjusted for creatinine (μg/g creatinine); † Birth weight < 2500 g regardless of gestational age at birth; [ᵞ] Any delivery before 37 weeks of gestation regardless of birth weight.

[a] Bivariate logistic regression; [b] Multivariate logistic regression adjusted for selenium concentration; [c] Multivariate logistic regression adjusted for maternal age, maternal education, parity, baby's sex, mode of delivery, smoking status and selenium concentration.

**Table 9a: Associations between Prenatal Arsenic Exposure and Newborn Leucocyte Telomere Length (n = 409)**

| Characteristics | Model 1 [a] | Model 2 [b] | Model 3 [c] |
|---|---|---|---|
| | Coefficient (95% CI) | Coefficient (95% CI) | Coefficient (95% CI) |
| **Arsenic concentration** [θ] | | | |
| Quartile 1 (< 45.5) | Ref | ref | ref |
| Quartile 2 (45.5 – 74.2) | - 0.07 (- 0.15, 0.19) | - 0.06 (- 0.15, 0.26) | - 0.06 (- 0.15, 0.03) |
| Quartile 3 (74.2 – 126.7) | - 0.14 (- 0.22, - 0.05) ** | - 0.12 (- 0.21, - 0.03) ** | - 0.11 (- 0.20, - 0.02) ** |
| Quartile 4 (> 126.7) | - 0.17 (- 0.26, - 0.08) *** | - 0.15 (- 0.24, - 0.05) ** | - 0.13 (- 0.22, - 0.03) ** |
| **Selenium concentration** [θ] | | | |
| Quartile 1 (< 17.8) | | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.03 (- 0.06, 0.12) | 0.03 (- 0.06, 0.12) |
| Quartile 3 (22.6 – 29.8) | | - 0.03 (- 0.12, 0.06) | - 0.03 (- 0.12, 0.06) |
| Quartile 4 (> 29.8) | | - 0.06 (- 0.15, 0.04) | - 0.05 (- 0.15, 0.04) |
| **Maternal age** (years) | | | - 0.004 (- 0.01, 0.002) |
| **Maternal education** | | | 0.005 (- 0.02, 0.03) |
| **Gestational age** (weeks) | | | - 0.003 (- 0.02, 0.01) |
| **Birth weight** (g) | | | 0.00 (- 0.00, 0.00) |
| **Primigravida** (ref: non primigravida) | | | - 0.03 (- 0.11, 0.05) |
| **Ethnicity** (ref: Bamar) | | | 0.01 (- 0.07, 0.05) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | - 0.03 (- 0.10, 0.04) |
| **Baby's sex** (ref: male) | | | - 0.03 (- 0.09, 0.03) |
| **Smoking status** (ref: no exposure) | | | - 0.01 (- 0.07, 0.05) |

** $p < 0.01$. ***$p < 0.001$

[θ] Adjusted for creatinine (μg/g creatinine)

[a] Bivariate linear regression; [b] Multivariate linear regression adjusted for selenium concentration; [c] Multivariate linear regression adjusted for maternal age, maternal education, gestational age, birth weight, parity, ethnicity, baby's sex, mode of delivery, smoking status, cadmium, lead and selenium concentration.

**Table 9b: Associations between Prenatal Cadmium Exposure and Newborn Leucocyte Telomere Length (n = 409)**

| Characteristics | Model 1 [a] Coefficient (95% CI) | Model 2 [b] Coefficient (95% CI) | Model 3 [c] Coefficient (95% CI) |
|---|---|---|---|
| **Cadmium concentration** [θ] | | | |
| Quartile 1 (< 0.5) | ref | ref | ref |
| Quartile 2 (0.5 – 0.9) | - 0.05 (- 0.13, 0.04) | - 0.04 (- 0.13, 0.05) | - 0.05 (- 0.14, 0.04) |
| Quartile 3 (0.9 – 1.4) | - 0.13 (- 0.22, - 0.04) ** | - 0.12 (- 0.21, - 0.03) ** | - 0.14 (- 0.23, - 0.04) ** |
| Quartile 4 (> 1.4) | - 0.19 (- 0.28, - 0.10) *** | - 0.17 (- 0.26, - 0.08) *** | - 0.19 (- 0.30, - 0.08) *** |
| **Selenium concentration** [θ] | | | |
| Quartile 1 (< 17.8) | | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.03 (- 0.05, 0.12) | 0.03 (- 0.06, 0.12) |
| Quartile 3 (22.6 – 29.8) | | - 0.01 (- 0.10, 0.08) | - 0.02 (- 0.11, 0.07) |
| Quartile 4 (> 29.8) | | - 0.04 (- 0.13, 0.06) | - 0.03 (- 0.13, 0.06) |
| **Maternal age** (years) | | | 0.002 (- 0.004, 0.01) |
| **Maternal education** | | | 0.00 (- 0.03, 0.03) |
| **Gestational age** (weeks) | | | - 0.00 (- 0.02, 0.02) |
| **Birth weight** (g) | | | - 0.00 (- 0.00, 0.00) |
| **Primigravida** (ref: non primigravida) | | | - 0.02 (- 0.10, 0.06) |
| **Ethnicity** (ref: Bamar) | | | 0.02 (- 0.05, 0.08) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | - 0.02 (- 0.09, 0.04) |
| **Baby's sex** (ref: male) | | | - 0.01 (- 0.08, 0.05) |
| **Smoking status** (ref: no exposure) | | | - 0.01 (- 0.05, 0.08) |

** $p < 0.01$. ***$p < 0.001$

[θ] Adjusted for creatinine (μg/g creatinine)

[a] Bivariate linear regression; [b] Multivariate linear regression adjusted for selenium concentration; [c] Multivariate linear regression adjusted for maternal age, maternal education, gestational age, birth weight, parity, ethnicity, baby's sex, mode of delivery, smoking status and selenium concentration.

**Table 9c: Associations between Prenatal Lead Exposure and Newborn Leucocyte TL (n = 409)**

| Characteristics | Model 1 [a] Coefficient (95% CI) | Model 2 [b] Coefficient (95% CI) | Model 3 [c] Coefficient (95% CI) |
|---|---|---|---|
| **Lead concentration** [θ] | | | |
| Quartile 1 (< 1.0) | ref | ref | ref |
| Quartile 2 (1.0 – 1.8) | - 0.10 (- 0.19, - 0.01) * | - 0.09 (- 0.18, - 0.00) * | - 0.09 (- 0.18, - 0.00) * |
| Quartile 3 (1.8 – 3.4) | - 0.04 (- 0.13, 0.04) | - 0.04 (- 0.13, 0.05) | - 0.04 (- 0.13, 0.05) |
| Quartile 4 (> 3.4) | - 0.14 (- 0.23, - 0.05) ** | - 0.11 (- 0.20, - 0.02) * | - 0.11 (- 0.20, - 0.02) * |
| **Selenium concentration** [θ] | | | |
| Quartile 1 (< 17.8) | | ref | ref |
| Quartile 2 (17.8 – 22.6) | | 0.02 (- 0.07, 0.11) | 0.01 (- 0.08, 0.10) |
| Quartile 3 (22.6 – 29.8) | | - 0.04 (- 0.13, 0.05) | - 0.05 (- 0.14, 0.05) |
| Quartile 4 (> 29.8) | | - 0.08 (- 0.17, 0.01) | - 0.08 (- 0.17, 0.01) |
| **Maternal age** (years) | | | - 0.002 (- 0.01, 0.004) |
| **Maternal education** | | | 0.01 (- 0.02, 0.04) |
| **Gestational age** (weeks) | | | - 0.001 (- 0.02, 0.02) |
| **Birth weight** (g) | | | 0.00 (- 0.00, 0.00) |
| **Primigravida** (ref: non primigravida) | | | - 0.02 (- 0.10, 0.06) |
| **Ethnicity** (ref: Bamar) | | | 0.02 (- 0.05, 0.09) |
| **Mode of delivery** (ref: normal vaginal delivery) | | | - 0.03 (- 0.10, 0.03) |
| **Baby's sex** (ref: male) | | | - 0.03 (- 0.09, 0.04) |
| **Smoking status** (ref: no exposure) | | | - 0.00 (- 0.07, 0.06) |

*$p < 0.05$. ** $p < 0.01$.

[θ] Adjusted for creatinine (μg/g creatinine)

[a] Bivariate linear regression; [b] Multivariate linear regression adjusted for selenium concentration; [c] Multivariate linear regression adjusted for maternal age, maternal education, gestational age, birth weight, parity, ethnicity, baby's sex, mode of delivery, smoking status and selenium concentration.

**Table 10: Associations between Adverse Birth Outcomes and Newborn Leucocyte Telomere Length (n = 409)**

| Characteristics | Low Birth Weight † | | Preterm ᵞ | |
|---|---|---|---|---|
| | OR (95% CI) | AOR (95% CI) | OR (95% CI) | AOR (95% CI) |
| Relative TLᶿ (T/S ratio) | 0.73 (0.21, 2.48) | 0.76 (0.22, 2.69) | 1.40 (0.65, 2.99) | 1.34 (0.60, 3.03) |
| Maternal age (years) | | 1.05 (0.97, 1.13) | | 1.03 (0.99, 1.08) |
| Maternal education | | 0.69 (0.44, 1.03) | | 0.89 (0.70, 1.14) |
| Primigravida (ref: non primigravida) | | 4.07 (1.31, 12.66) * | | 1.76 (0.91, 3.38) |
| Antenatal visits ≥ 4 times (ref: < 4 times) | | 0.62 (0.26, 1.45) | | 1.45 (0.82, 2.56) |
| Mode of delivery (ref: normal vaginal delivery) | | 0.61 (0.26, 1.41) | | 0.37 (0.22, 0.63) *** |
| Baby's sex (ref: male) | | 1.43 (0.62, 3.28) | | 1.18 (0.71, 1.96) |
| Smoking status (ref: no exposure) | | 0.85 (0.37, 1.96) | | 0.85 (0.37, 1.96) |

*$p < 0.05$. ***$p < 0.001$.

ᶿ Log-transformed value.

† Birth weight < 2500 g regardless of gestational age at birth.

ᵞ Any delivery before 37 weeks of gestation regardless of birth weight.

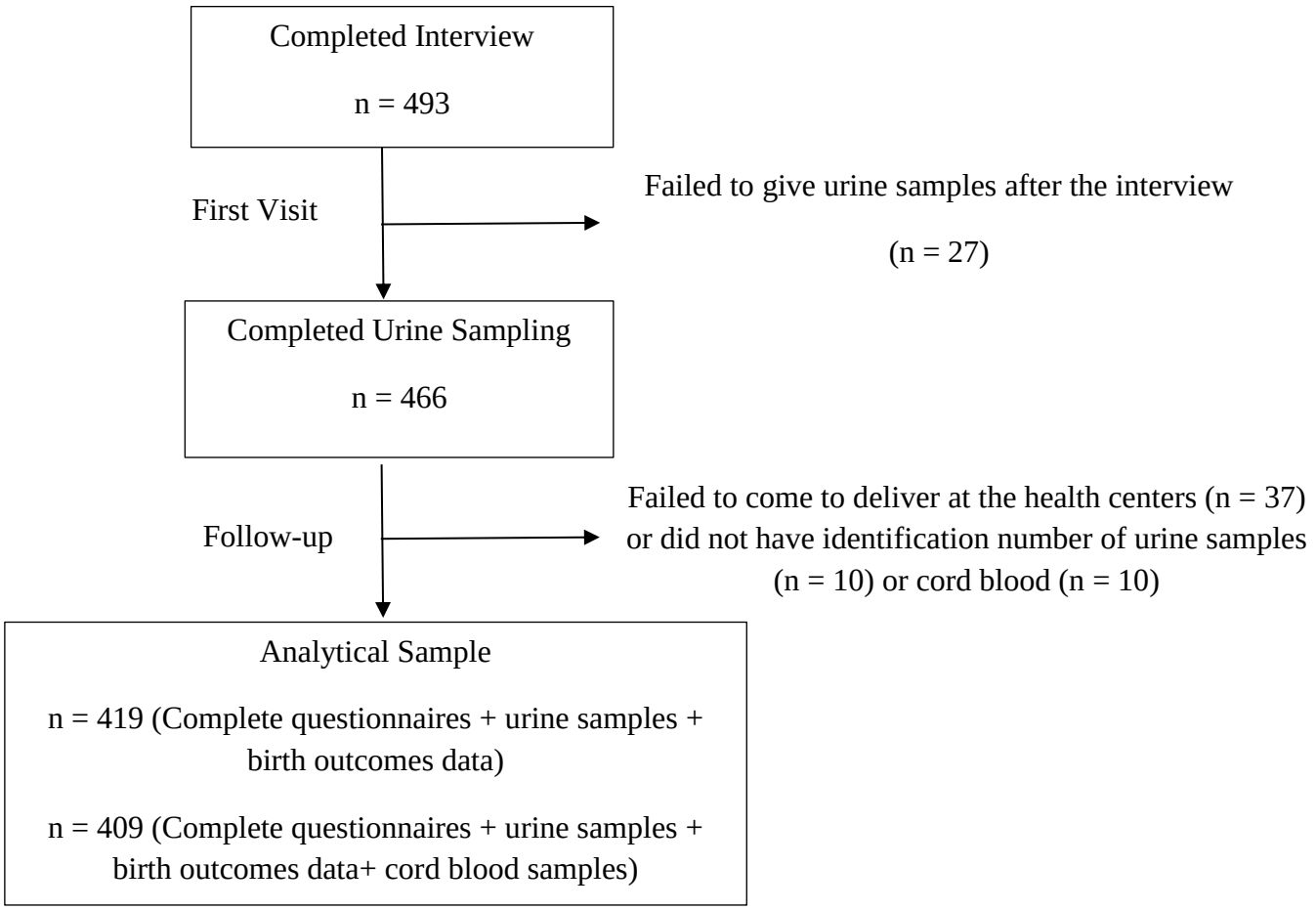
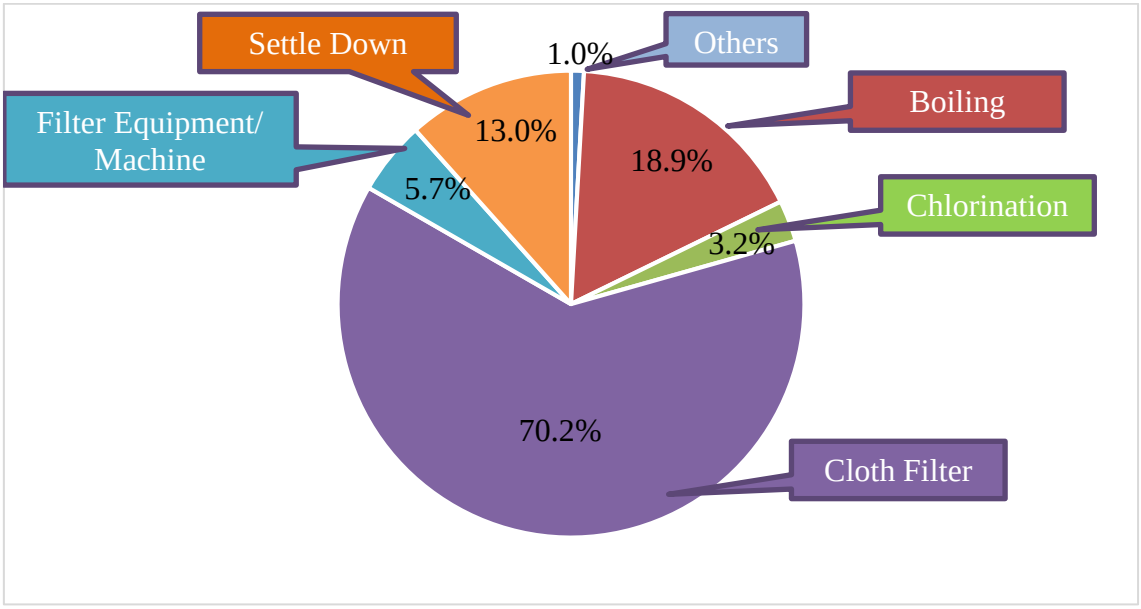


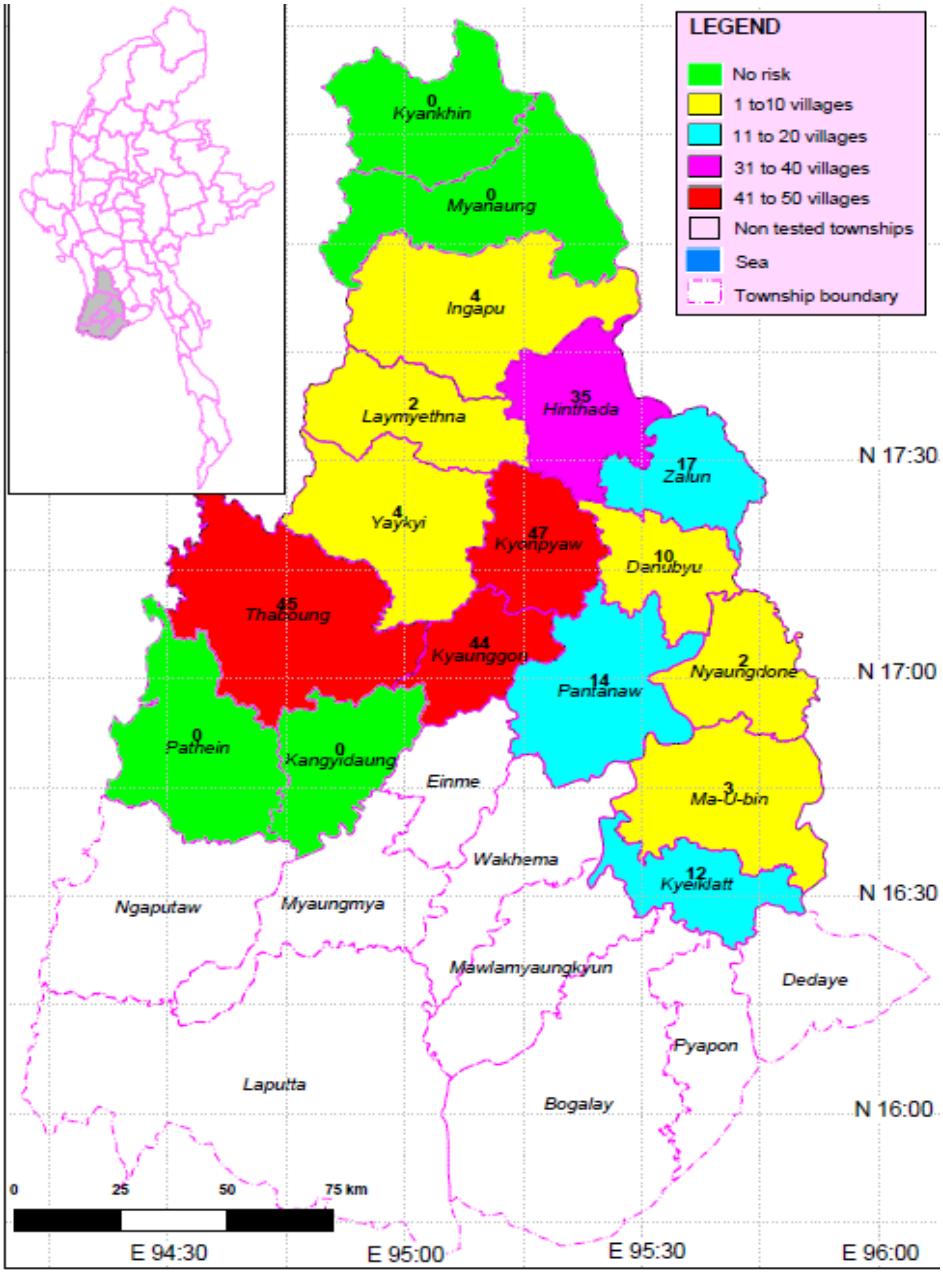

**Figure 2: Participation Flow Chart of the Study**




[θ] Multiple responses were allowed.

**Figure 3: Methods of Household Drinking Water Treatment [θ] (n = 414)**

**Appendix 1: Map of Study Area**




Source: UNICEF and MOH, 2013

**Appendix 2: Questionnaire (English Version)**

**SURVEY QUESTIONNAIRE (English version)**

**Identification:**

| **Township:** | **Ward name:** |
|---|---|
| **Interviewer**<br>**Name:** | **Date of interview:** / / |

| **Field Editor** | **Data Entry** |
|---|---|
| Name:<br>Date: / / | Name:<br>Date: / / |

**Introduction**

Mingalarbar!

My name is . We are interviewing pregnant women in their third trimesters about the drinking water status and pregnancy and child birth. The information you give to me is confidential. The result of this survey will be used to help improve health programs for women.

**Eligibility**

Pregnant women (aged 18 years or above) in their third trimesters, residing in the study area for more than six months.

**Instruction: Circle the answers, which are responded by the participants or fill in the blank areas. Thank you for your participation.**

## Part (A)

### 1. Socio-demographic characteristics

| No | Questions | Answers | Codes | Note |
|---|---|---|---|---|
| 1. | In what month and year were you born? | ……. Month<br>……. Year<br>Don't know | <br><br>99 | |
| 2. | How old are you? (Completed year) | | | |
| 3. | What is your religion? | Buddhist | 1 | |
| | | Christian | 2 | |
| | | Muslim | 3 | |
| | | Hindu | 4 | |
| | | Others | 5 | |
| 4. | What is your ethnicity? | Bamar | 1 | |
| | | National races | 2 | |
| | | Others | 3 | |
| 5. | What is the highest education or year of school you completed? | Illiterate | 1 | |
| | | Read and write | 2 | |
| | | Primary school completed | 3 | |
| | | Middle school completed | 4 | |
| | | High school completed | 5 | |
| | | Graduate and above | 6 | |
| 6. | What is your main occupation? | Unemployed | 1 | |
| | | House-wife | 2 | |
| | | Farmers | 3 | |

| | | | | |
|---|---|---|---|---|
| | | Private sectors | 4 | |
| | | Government servants | 5 | |
| | | Own business | 6 | |
| | | Others | 7 | |
| 7. | Do you or have you worked for any of the following? | Armed force | 1 | Multiple responses are possible |
| | | Glass industry | 2 | |
| | | Coal mine/Refinery | 3 | |
| | | Saw mill | 4 | |
| | | Cotton field/Orchid | 5 | |
| | | Mining/Smelting | 6 | |
| | | Electronic plants | 7 | |
| | | None of these | 8 | |
| 8. | If yes, how long do you or have you worked? | For ……<br>At …… | | |
| 9. | How much is monthly household income? | In local currency (kyats)<br>…… | | Skip if the respondent is unemployed |

**2. Assessment of Quality of Drinking Water**

| No | Questions | Answers | Codes | Note |
|---|---|---|---|---|
| 1. | Which of the following sources of drinking water are available in your neighborhood? | Bore well/Hand pump/ Motor pump | 1 | Multiple responses are possible |
| | | Community well | 2 | |
| | | River/Lake water | 3 | |
| | | Household water supply (Municipal piped) | 4 | |
| | | Rain water | 5 | |
| | | Others (Specify) | 6 | |
| 2. | What is your primary source of drinking water? | Bore well/Hand pump/ Motor pump | 1 | Single response |
| | | Community well | 2 | |
| | | River/Lake water | 3 | |
| | | Household water supply (piped) | 4 | |
| | | Rain water | 5 | |
| | | Others (Specify) | 6 | |
| 3. | How long it had been as the primary drinking water source? | …… | | If the dwelling spot is changed, please count the duration from the new dwell. |
| 4. | If the primary source of drinking water is shallow or | …….. | | |

| | | | | |
|---|---|---|---|---|
| | deep well, please mention how deep is it? | Don't know | 99 | |
| 5. | Do you treat your water in any way to make it safer to drink? | No | 0 | If No, skip to question no 6. |
| | | Yes | 1 | |
| 6. | If yes, how often do you treat? | Always | 1 | |
| | | Usually | 2 | |
| | | Sometimes | 3 | |
| | | Few | 4 | |
| 7. | If yes, what do you usually do your water to make it safer to drink? | Boil | 1 | Multiple responses are possible |
| | | Add bleach/Chlorine | 2 | |
| | | Strain it through a cloth | 3 | |
| | | Use a water filter (ceramics, sand, composite, etc.) | 4 | |
| | | Solar disinfection | 5 | |
| | | Let it stand and settle | 6 | |
| | | Others (Specify) | 7 | |
| 8.. | When was the last time the water was tested? | < 1month | | If Never skip to question no 9. |
| | | Within 1- 3 months | | |
| | | Within 3 - 6 months | | |
| | | More than 6 months | | |
| | | Never | | |
| 9.. | What was it tested for? | Bacteria | 1 | |
| | | Chemicals | 2 | |
| | | Others | 3 | |
| 10. | Who (Which organization) did the testing? | Government Organizations | 1 | |

| | | | | |
|---|---|---|---|---|
| | | NGOs/NPOs | 2 | |
| | | Other | 3 | |
| 11. | Has it ever been tested for arsenic? | No | 0 | |
| | | Yes | 1 | |
| 12.. | If yes, what were the results? | Above standard (> 10 µg/L) | 1 | |
| | | Below standard (<= 10 µg/L) | 2 | |
| | | Don't know | 3 | |

### 3. Food Consumption

| No. | Questions | Answers | Codes | Note |
|---|---|---|---|---|
| How often, in the past 3 months, did you eat the following? | | | | |
| 1. | Seafood | Never | 1 | |
| | | < 1 time per week | 2 | |
| | | 1 - 6 times per week | 3 | |
| | | 1 - 3 times per day | 4 | |
| | | ≥ 4 times per day | 5 | |
| 2. | Rice or other cooked grains including brown rice, cracked wheat and millet (per 3 meals in a day) | Almost never or never | 1 | |
| | | About ⅓ of the time | 2 | |
| | | About ⅔ of the time | 3 | |
| | | Almost always or always | 4 | |
| 3. | Ways of rice cooking | Traditional boiling method | 1 | |
| | | Absorption method (by rice cooker) | 2 | |
| | | Others | 3 | |

**4. Maternal Health during Pregnancy**

| No. | Questions | Answers | Codes | Note |
|---|---|---|---|---|
| 1. | How many months pregnant are you? | …….. months | | |
| 2. | Is this your first pregnancy? | No | 0 | |
| | | Yes | 1 | |
| 3. | If no, how many of pregnancies resulted in a baby that was born alive or born dead? | Baby born alive ….<br>Baby born dead ….<br>Abortion…….. | | |
| 4. | Did your most recent birth result in a baby that was born alive or dead? | Live birth | 1 | |
| | | Stillbirth | 2 | |
| 5. | If live birth, in which month and year did your most recent birth occur? | | | |
| 6. | How many times in total have you received antenatal care during your current pregnancy? | …….. | | |
| 7. | How many months pregnant were you when you first received antenatal care for this pregnancy? | …….. | | |
| 8. | Whom did you first see for checkup on your current pregnancy? | Doctor | 1 | |
| | | Nurse/Midwife | 2 | |
| | | TBA/Traditional healer | 3 | |
| | | Community health worker | 4 | |
| | | Relative/Friend | 5 | |
| | | Others | 6 | |

| | | | | | |
|---|---|---|---|---|---|
| 9. | Which arrangement have your or your family made for the birth of this child. | | No | Yes | |
| | | Transport | 0 | 1 | |
| | | Save money | 0 | 1 | |
| | | A blood donor | 0 | 1 | |
| | | A skilled provider | 0 | 1 | |
| | | A safe delivery place | 0 | 1 | |
| | | Others (Specify) | 0 | 1 | |
| 10. | During the antenatal period, did you experience any serious health problems? | No | 0 | | |
| | | Yes | 1 | | |
| 11. | If yes, what problems did you experience? | Severe vaginal bleeding | 1 | | |
| | | Severe vaginal discharge | 2 | | |
| | | Convulsion | 3 | | |
| | | High fever | 4 | | |
| | | Loss of consciousness | 5 | | |
| | | Swollen hands/face | 6 | | |
| | | Severe headache | 7 | | |
| | | Severe abdominal pain | 8 | | |
| | | Water breaks without labor | 9 | | |
| | | Accelerated/reduced fetal movement | 10 | | |
| | | Others(Specify) | 99 | | |
| 12. | Are you suffering any underlying medical disease? | No | 0 | | If yes, state. …… |
| | | Yes | 1 | | |
| 13. | Are you currently taking any medication? | No | 0 | | If yes, state. …… |
| | | Yes | 1 | | |

| | | | | | |
|---|---|---|---|---|---|
| 14. | How is your smoking status? | | No | Yes | |
| | | Smoke before pregnancy | 0 | 1 | |
| | | Smoke during pregnancy | 0 | 1 | |
| | | Second hand exposure to smoking | 0 | 1 | |
| | | Never smoke or being exposed | 0 | 1 | |
| 15. | How is your alcohol drinking status? | | No | Yes | |
| | | Drink before pregnancy | 0 | 1 | |
| | | Drink during pregnancy | 0 | 1 | |
| | | Never drink | 0 | 1 | |
| Clinical and Laboratory Information from Antenatal Record | | | | | |
| 16. | Body Weight | | | | |
| 17. | Height | | | | |
| 18. | Temperature | | | | |
| 19. | Edema | | | | |
| 20. | Pulse | | | | |
| 21. | Blood Pressure | | | | |
| 22. | Hemoglobin | | | | |
| 23. | Urine test (AI/sugar) | | | | |
| 24. | Malaria | | | | |
| 25. | Prophylactic Treatment | | No | Yes | |
| | | Tetanus toxoid | 0 | 1 | |
| | | Iron | 0 | 1 | |
| | | Folic acid | 0 | 1 | |
| | | Vitamin B1 (B complex) | 0 | 1 | |
| 26. | Any diagnosed pregnancy related complication | …… | | | |

**Part (B)**

**Identification:**

| **Township:** | **Ward name:** |
|---|---|
| **Interviewer**<br>**Name:** | **Date of interview:** / / |

| **Field Editor** | **Data Entry** |
|---|---|
| Name:<br>Date: / / | Name:<br>Date: / / |

**5. Delivery Record of Mothers and Newborns**

| **No.** | **Questions** | **Answers** | **Codes** | **Note** |
|---|---|---|---|---|
| **Maternal Information** | | | | |
| 1. | Admission Date | | | |
| 2. | Contraction Started | Date<br>Time | | |
| 3. | Maternal weight | ……. (kg) | | |
| 4. | Maternal height | …….. ($m^2$) | | |
| 5. | Obstetric complications during pregnancy | Antenatal hemorrhage | 1 | |
| | | Postpartum hemorrhage | 2 | |
| | | Postpartum sepsis | 3 | |
| | | Severe pre-eclampsia/Eclampsia | 4 | |
| | | Ruptured uterus | 5 | |
| | | Labor lasting >12 hours | 6 | |
| | | Placenta not delivered 30 minutes after baby | 7 | |
| | | Others (Specify) | 99 | |

| | | | | |
|---|---|---|---|---|
| | | Nil | 98 | |
| 6. | Any referral history | No | 0 | |
| | | Yes | 1 | |
| 7. | Reason of referral | ……. | | |
| **Newborns Information** | | | | |
| 6. | Date of Birth | | | |
| 7. | Sex | | | |
| 8. | Birth weight | | | |
| 9. | Height | | | |
| 10. | Head Circumference | | | |
| 11. | APGAR score | | | |
| 12. | Type of Delivery | Normal spontaneous vaginal delivery | 1 | |
| | | Assistant delivery (vacuum extraction or forceps) | 2 | |
| | | Cesarean section | 3 | |
| 13. | Presentation | ……. | | |
| 14. | Delivery Outcomes | Live birth | 1 | |
| | | Stillbirth | 2 | |
| | | IUFD | 3 | |
| | | Premature | 4 | |
| | | Twins | 5 | |
| | | Congenital abnormalities | 6 | |
| | | Others | 7 | |
| 15. | Baby to Breast/ Suck | Within 1 hour | 1 | |
| | | > 1 hour | 2 | |
| | | None | 3 | |

**Thank you so much for your cooperation.**

**Appendix 3: Instruction for Urine Sampling**

Maternal spot urine is collected at the time of antenatal visit at third trimesters by the research team.

1) Collect spot urine of about 5 – 20 mL from the pregnant women.
2) Fasting or special diet is not necessarily to be requested.
3) Urine samples are initially collected in storage 60 mL plastic cabinet and then transfer to 5 mL polyethylene tubes with airtight lids.
4) Avoid contact with the inside or refrain from leaving the tubes open to air longer than necessary to minimize the external contamination.
5) Label identification number and keep frozen immediately.

**Appendix 4: Instructions for Cord Blood Sampling**

The fetal cord blood is to be collected shortly after the delivery under aseptic conditions. Procedures are almost the same for non-cesarean section and cesarean section.

1) A segment of cord must be separated between two sets of clamps immediately after delivery as normal procedure permits.
2) Ensure that the cord segment is full of blood. If needed, perform milking of the cord before clamping.
3) Recommended for a segment of cord (about 8 inches) be separated from the rest of the cord and placenta to allow the third stage to continue without further interruption.
4) Choose a site 4 - 6 inches from the cut end of umbilical cord for the cord blood sampling.
5) Clean the chosen site using septic swabs, wiping the entire width of cord about 4 inches nearby the in-situ site.
6) Using the sterile syringe, insert the needle into the chosen (cleaned) site of umbilical vein of cord and draw about 5 mL of cord blood.
7) Transfer the collected blood into the provided vacuum tubes containing anticoagulant.
8) Proper labeling of respective identification numbers and keep frozen within 1 hour.

## Appendix 5: Ethical Approval Letter (the University of Tokyo)

**Graduate School of Medicine and Faculty of Medicine**
**The University of Tokyo**
**7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan**

**Ethics Committee**

Date: July 11th, 2016

Serial Number: 11186

Title of research: Arsenic Contamination in Drinking Water and Its Health Outcomes Among Pregnant Women and Newborns in Ayeyarwaddy Region, Myanmar

Name of applicant: Chiho Watanabe, Professor,
Departmeny of Human Ecology,
Graduate school of Medicine, The University of Tokyo

This is to certify that a plan for the research project identified above was reviewed, and was approved by the Ethics Committee on June 6th, 2016.

THE UNIVERSITY OF TOKYO

Kohei Miyazono, Dean
Graduate School of Medicine and
Faculty of Medicine
The University of Tokyo

KM/tu

東京大学
THE UNIVERSITY OF TOKYO

## Appendix 6: Ethical Approval Letter (Department of Medical Research, Myanmar)

The Government of the Republic of the Union of Myanmar
Ministry of Health and Sports
Department of Medical Research
No. 5, Ziwaka Road, Dagon Township, Yangon 11191
Tel : 95-1-375447, 95-1-375457, 95-1-375459 Fax : 95-1-251514

ERC Number: 009316
Approval Number: Ethics/DMR/2016/110
Date of Approval: 9 September, 2016 (valid up to 8 September, 2017)

Project Title: **Arsenic Contamination in Drinking Water and its Health Outcomes Among Pregnant Women and Newborns in Ayeyawaddy Region, Myanmar**

Principal Investigator: Dr. Kyi Mar Wai
The University of Tokyo

Documents Accepted:

1. Ethical Proposal Form version 2.0 Dated 22 June, 2016
2. Full Proposal Protocol version 2.0 Dated 22 June, 2016
3. Proposal Summary version 2.0 Dated 22 June, 2016
4. Agreement to comply with ethical guideline version 1.0 Dated 22 June, 2016
5. Informed Consent Form (English & Myanmar) version 2.0 Dated 22 June, 2016
6. Information sheet for taking biological specimen (English & Myanmar) version 2.0 Dated 22 June, 2016
7. Questionnaires (English & Myanmar) version 2.0 Dated 22 June, 2016
8. Approval from Ethics Committee, The University of Tokyo Dated 11 July, 2016
9. Material Transfer Agreement version 1.0 Dated 9 August, 2016
10. Investigators' CV version 2.0 Dated 22 June, 2016

The Ethics Review Committee on Medical Research Involving Human Subjects, Department of Medical Research, Ministry of Health and Sports approves to conduct the proposed research project as it is in full compliance with the Declaration of Helsinki, Council for International Organizations of Medical Sciences guidelines and International Conference on Harmonisation in Good Clinical Practice guidelines.

**Prof. Pe Thet Khin**
**Chairperson**
**Ethics Review Committee**
**Department of Medical Research**

IORG Number: IORG0007357 FWA Number: FWA00018816 IRB Number: IRB00008835

## Appendix 7: Material Transfer Agreement

**Ethics Review Committee**
**Department of Medical Research**
**Ministry of Health and Sports**
**Republic of the Union of Myanmar**

**Material Transfer Agreement**

**Research study title**: Arsenic Contamination in Drinking Water and its Health Outcomes Among Pregnant Women and Newborns in Ayeyarwaddy Region, Myanmar.
This Material Transfer Agreement is made by and between the University of Medicine (1) **(Prof. Ohnmar)** and The University of Tokyo **(Prof. Chiho Watanabe and Dr. Kyi Mar Wai)** hereby agree to the following terms and conditions:

1. The biological materials (**material**) to be provided to **recipient** are drinking water, maternal urine and cord blood.
2. The material shall be used exclusively for non-commercial, non-military scientific research by the **recipient**. The materials shall be used only at the **recipient** organization and only in the **recipient** scientist's laboratory under the direction of the **recipient** scientist or others working under his/her direct supervision.
3. The materials are the property of the **provider**. Ownership of modifications and direct/indirect derivatives of materials, and income arising from commercializing the direct/indirect derivatives of the materials will be negotiated in good faith by the parties hereto depending upon (a) their relative contribution to the creation of said modifications and derivatives, and (b) applicable laws and regulations.
4. **Recipient** shall not sell or otherwise distribute the materials to a third party for any purpose. This agreement and the resulting transfer of materials constitute a non-exclusive license to use the materials solely for research or other not-for-profit purpose and specifically as described in the study stated above.
5. **Recipient** shall consult with the **provider** prior to the preparation and submission of presentation/publication materials and patent applications, which involve the materials, modification of materials and direct/indirect derivatives of materials.
6. **Recipient** agrees to provide the **provider** with a copy of any publication, which contains experimental results obtained from the use of the materials, modification of materials and direct/indirect derivatives of materials. The **recipient** shall recognize the **provider** as co-author in all publications containing any data or information about the materials, modification of materials and direct/indirect derivatives of materials unless the **provider** indicates otherwise.
7. This Agreement will terminate on the earliest of the following dates:
   a) When the material becomes generally available from third parties, for example, through reagent catalogs or public depositories or
   b) On completion of the current research with the **material**, or

c) On thirty (30) days written notice by either party to the other, or

d) On the date specified in an implementing letter, provided that:

i) if termination should occur under 7(a), the **recipient** shall be bound to the **provider** by the least restrictive terms applicable to the **material** obtained from the then-available resources; and

ii) if termination should occur under 7(b) or (d) above, the **recipient**, will discontinue its use of the material and will, upon direction of the **provider**, return or destroy any remaining material. The **recipient**, at its discretion, will also either destroy the modifications or remain bound by the terms of this agreement; and

iii) in the event the **provider** terminates this Agreement under 7 (c) other than for breach of this agreement or for cause such as an imminent health risk or patent infringement, the **provider** will defer the effective date of termination for a period of up to one year, upon request from the **recipient**, to permit completion of research in progress. Upon the effective date of termination, or if requested, the deferred effective date of termination, **recipient** will discontinue its use of the **material** and will, upon direction of the **provider**, return or destroy any remaining material. The **recipient**, at its discretion, will also either destroy the modifications or remain bound by the terms of this agreement as they apply to modifications.

Accepted by:

PROVIDER SCIENTIST

Signature and Date: 9 Aug 2016
Printed Name: Professor Ohnmar
Designation: Department of Physiology, The University of Medicine (1), Ministry of Health and Sports, Myanmar

RECIPIENT SCIENTIST

Signature and Date: 4th Aug, 2016
Printed Name: Dr. Kyi Mar Wai
Designation: Department of Human Ecology, School of International Health, Graduate School of Medicine, the University of Tokyo

PROVIDER INSTITUTION APPROVAL

Signature and Date: 3/8/2016
Printed Name: Professor Zaw Wai Soe
Designation: Rector, The University of Medicine (1), Ministry of Health and Sports, Myanmar
Institution Seal

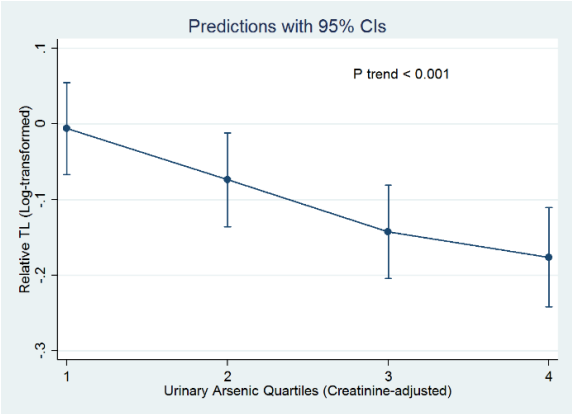
RECIPIENT INSTITUTION APPROVAL

Signature and Date: Aug 4, 2016
Printed Name: Professor. Chiho Watanabe
Designation: Department of Human Ecology, School of International Health, Graduate School of Medicine, the University of Tokyo
Institution Seal

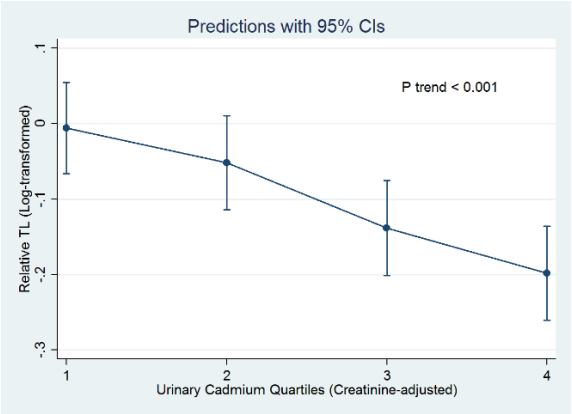
**Appendix 8: Arsenic Concentration in Drinking Water, Stratified by the WHO Guidelines (n = 248)**

| Arsenic Concentration | Number | Percentage |
|---|---|---|
| < 10 μg/L | 192 | 77.4 |
| ≥ 10 μg/L | 56 | 22.6 |

**Appendix 10: Newborn Leucocyte Telomere Length Across the Quartiles of (A) Arsenic and (B) Cadmium (n = 409) †**




(A)




(B)

† Quartile exposure levels (µg/g creatinine) for arsenic are 1st < 45.5, 2nd 45.5 – 74.2, 3rd 74.2 – 126.7 and 4th > 126.7, and for cadmium are 1st < 0.5, 2nd 0.5 – 0.9, 3rd 0.9 – 1.4 and 4th > 1.4.