MAHARASHTRA ANAEMIA STUDY: AN INVESTIGATION OF FACTORS ASSOCIATED WITH ADOLESCENT HEALTH AND PREGNANCY-RELATED OUTCOMES IN WOMEN FROM MAHARASHTRA STATE, INDIA

Submitted by
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I dedicate this thesis to adolescent girls and pregnant women from Osmanabad district of Maharashtra, India for providing their valuable time to conduct this research.

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List of Abbreviations

Hb	Haemoglobin
IDA	Iron Deficiency Anaemia
WHO	World Health Organisation
NFHS	National Family Health Survey
NRHM	National Rural Health Mission
PNDT	Pre-natal Diagnostic Techniques
PCPNDT	Pre-conception and Pre-natal Diagnostic Techniques
MAS	Maharashtra Anaemia Study
HMF	Halo Medical Foundation
UON	University of Nottingham
GBP	Great Britain Pound
INR	Indian Rupees
VHW	Village Health Worker
DHO	District Health Office
OR	Odds Ratio
CI	Confidence Intervals
β	Beta coefficient
g/dl	Grams per decilitre
RBC	Red Blood Cells
WBC	White Blood Cells
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
RDW	Red Cell Distribution Width
RBS	Random Blood Sugar

Synopsis

Maharashtra Anaemia study (MAS) was conducted as a part of the PhD programme of Dr Anand Ahankari through a joint collaboration of the University of Nottingham, UK and Halo Medical Foundation, India. The main goal of the study was to establish baseline epidemiological data for anaemia research in pregnant women and adolescent girls in Maharashtra state of India. Iron deficiency anaemia is the most common form of anaemia observed in India, and assessed based on haemoglobin (Hb) levels in blood. Clinically, anaemia is categorised in mild, moderate and severe form based on Hb levels.

The project had three main sections, a) Adolescent girls cross sectional survey, b) Pregnant women prospective study, and c) Maharashtra state birth registry analysis.

The study aimed to investigate individual and village level risk factors of anaemia in adolescent girls (13 to 17 years), and pregnant women (3 to 5 months) living in rural Maharashtra. Data from pregnant women were also used to examine risk factors associated with low birth weight (LBW). A recently introduced non-invasive haemoglobin (Hb) technology (known as NBM 200) was validated in this Indian setting by comparing Hb measurements obtained from the NBM 200 with reference blood measurements. In the adolescent survey, Sahli's hemometer (finger prick technique) was used to estimate reference Hb values, while in pregnant women venous blood samples were obtained to measure Hb using an automated analyser. Anaemia was defined using Hb levels based on the following cut offs, (a) Hb <12.0 g/dl in adolescent girls, and in (b) Hb <11.0 g/dl in pregnant women. Multivariable regression technique was used to identity risk factors associated with anaemia and LBW.
The Maharashtra state birth registry records covering a 32-year period (1980 to 2011) were investigated to assess temporal changes in the sex ratio at birth to investigate impacts of sex determination prevention legislations (known as PNDT 1994 and PCPNDT 2003).

The adolescent girls' survey showed a very high prevalence of anaemia (87%). Of 45 factors assessed in the survey, four were associated with adolescent anaemia. Anaemia likelihood increased significantly with age (Odds Ratio [OR] 1.41 per year, 95% CI: 1.17 to 1.70). Factors associated with decreased risk of anaemia were higher mid upper arm circumference (> 22 cm) (OR 0.51, 95% CI: 0.31 to 0.82), and ≥3 days/week consumption of fruit (OR 0.35, 95% CI: 0.23 to 0.54). At village level piped water supply was associated with higher Hb levels (β coefficient 0.61 g/dl, 95% CI: 0.39 to 0.82). Results from the NBM 200 reported wide agreement levels in the Bland-Altman analysis (mean difference of -2.70 g/dl, 95% CI: -2.84 to -2.55) demonstrating an overestimation of Hb by the NBM 200 compared to Sahli's hemometer. The NBM 200 showed low sensitivity (23.6%) and moderate specificity (61.8%) for the diagnosis of anaemia in the adolescent population.

Findings from pregnant women showed high anaemia prevalence (77%). Of 51 factors assessed in the study, three were associated with maternal anaemia. Increased risk of anaemia was seen in women with consanguineous marriages (OR 2.41, 95% CI: 1.16 to 5.01). Post-delivery data from full-term singleton live births showed the prevalence of LBW babies was 7%. Consanguineous marriage was a major risk of LBW babies in our study population (OR 5.68, 95% CI: 1.58 to 20.32). Village level risk factors showed lower likelihood of maternal anaemia with regular access to government nurses (OR 0.48, 95% CI: 0.25 to 0.93). The NBM 200 validation showed overestimation of Hb levels and underestimation of anaemia. Bland-Altman analysis showed a mean difference of -1.8 g/dl (95% CI: -2.06 to -1.71) indicating a systematic overestimation by the NBM 200 compared to venous Hb measurements. The device showed low sensitivity (33.7%) but high specificity (91.8%) for the diagnosis of anaemia in the pregnant woman population.

The 32 years of longitudinal birth registry data showed a significant increase in the sex ratio at live birth from 1980 to 2004, and then a subsequent decrease in sex ratio. The annual state male:female sex ratio of Maharashtra increased from a baseline of 1.11 in 1980 to a maximum value of 1.23 in 2003, before decreasing to 1.16 in 2011. This represented an increase in the annual sex ratio at live birth from 1980 to 2004 of 0.005 units per year ($p < 0.001$), and a decrease of 0.009 units per year after 2004 ($p < 0.01$). The increase in the sex ratio was consistent with the hypothesis of both increasing availability and acceptability of ultrasound scanning during this period, enabling foeticide of females in utero. The probable cause for the decrease in sex ratio after 2004 is likely to be due to the strengthening of the legislation banning sex-specific foeticide.

Published research papers from this PhD project (updated on 6th October 2017)

1. **Ahankari AS**, Myles PR, Dixit JV, Tata LJ, Fogarty AW. Risk factors for maternal anaemia and low birth weight in pregnant women living in rural India: a prospective cohort study. **Public Health** *July 2017*. doi: 10.1016/j.puhe.2017.06.023.

2. **Ahankari AS,** Fogarty AW, Tata LJ, Myles PR. Healthcare benefits linked with Below Poverty Line registration in India: Observations from Maharashtra Anaemia Study (MAS). **F1000Research** *January 2017*. doi: 10.12688/f1000research.10556.1

3. **Ahankari AS**, Myles PR, Tata LJ, Fogarty AW. PCPNDT seems to benefit India's birth sex ratios. **BMJ** *September 2016.* doi: 10.1136/bmj.i5194.

4. **Ahankari AS**, Myles PR, Fogarty AW, Dixit JV, Tata LJ. Prevalence of iron deficiency anaemia and risk factors in 1,010 adolescent girls from rural Maharashtra, India: a cross-sectional survey. **Public Health** *August 2016*. doi: 10.1016/j.puhe.2016.07.010.

5. **Ahankari AS**, Dixit JV, Fogarty AW, Tata LJ, Myles PR. Comparison of the NBM 200 non-invasive haemoglobin sensor with Sahli's hemometer among adolescent girls in rural India. **BMJ Innovations** *August 2016*. doi: 10.1136/bmjinnov-2015-000139.

6. **Ahankari AS,** Fogarty AW, Tata LJ, Dixit JV, Myles PR. Assessment of a non-invasive haemoglobin sensor NBM 200 among pregnant women in rural India. **BMJ Innovations** *February 2016*. doi: 10.1136/bmjinnov-2015-000085.

7. **Ahankari AS,** Myles PR, Tata LJ, Fogarty AW. Banning of fetal sex determination and changes in sex ratio in India. **Lancet Global Health** *September 2015*. doi: 10.1016/S2214-109X(15)00053-4.

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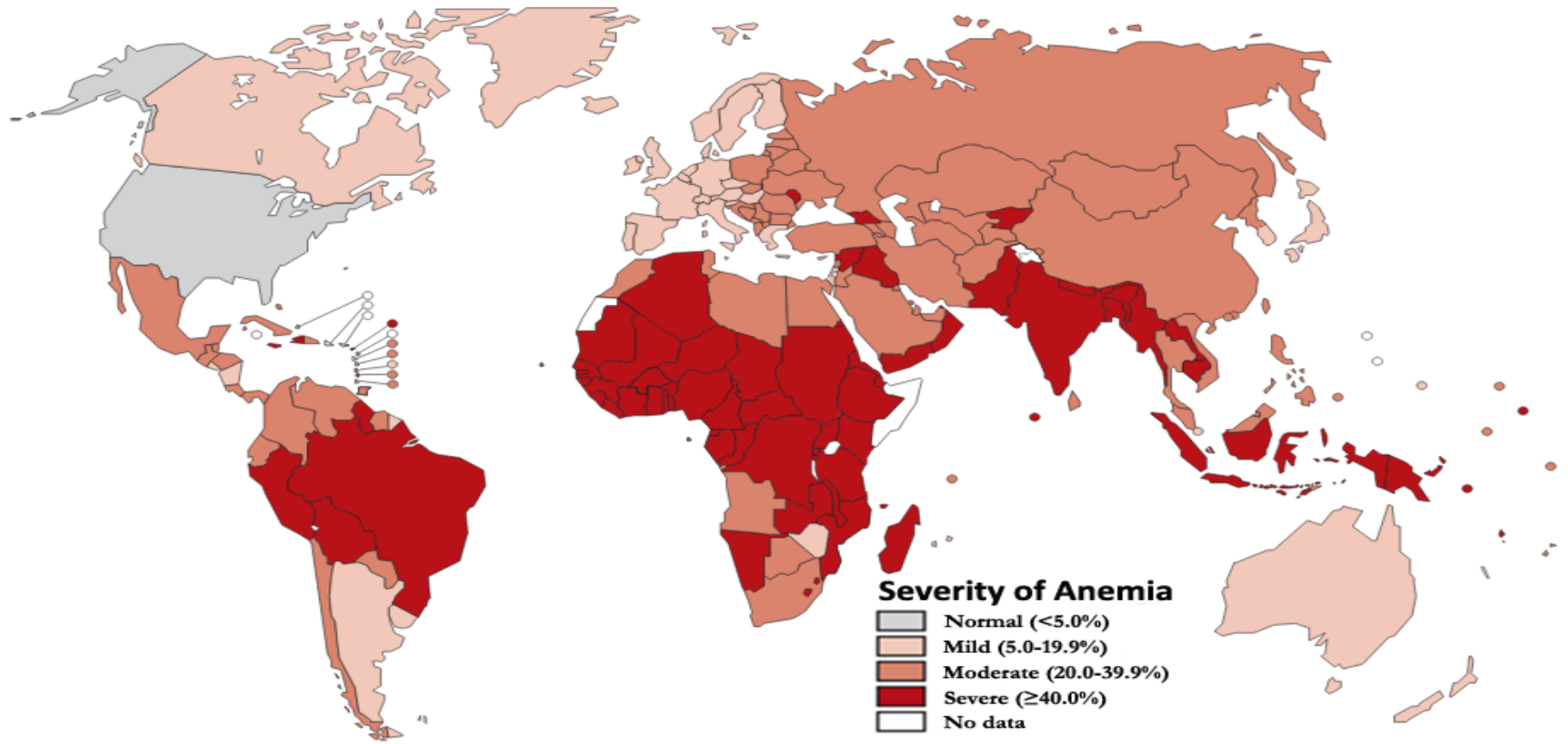
Chapter 1. Introduction

1.1 Anaemia

Anaemia is a condition in which the number of red blood cells or their capacity to carry oxygen reduces due to a variety of reasons such as pregnancy, nutritional deficiencies or diseases like malaria [1]. Globally two billion people are affected by anaemia [1]. The majority of anaemia cases (approximately 90%) are from developing countries in South East Asia and Sub Saharan Africa (Figure 1) [2]. A recent analysis of global data indicated that about 33% of the world's population is anaemic, which is equivalent to 68 million disability adjusted life years [3]. The global prevalence has decreased among males, however high prevalence is consistently recorded in women and children since 1990 [3].

Anaemia in adolescent girls and pregnant women is a major health concern. Anaemia during childhood affects physical growth, and later during adolescence age it may affect school performance, social development, and general wellbeing [4]. If untreated, it impacts on the health of the pregnant woman and her baby, for example maternal anaemia can lead to poor pregnancy outcomes pre-term delivery and low birth weight [5]. Globally, about 20 million low birth weight (< 2.5 Kg at birth) babies are born with a higher likelihood of childhood infections, and higher risks of acquiring non-communicable diseases compared to babies with healthy birth weight (> 2.5 Kg at birth) [6,7]. Therefore, anaemia is an important public health issue that demands research, particularly in developing countries where high prevalence is consistently documented.

Figure 1: Global prevalence of anaemia (countries with more than 40% of prevalence are indicated as severe)



Source of figure: Knowledge for Health, Johns Hopkins Centre for Communication Programs, USA supported by USAID.

1.2 Disease background

Anaemia is a systemic condition, that affects red blood cell (RBC) production and may alter cell characteristics such as shape, which in turn reduces the oxygen carrying capacity [8]. Effects of anaemia may vary with gender, age, pregnancy status and altitude. Broadly there are three main aetiological factors: anaemia due to (a) blood loss, (b) impaired production of RBCs and (c) increased destruction of RBCs. The most common cause is impaired production due to iron deficiency [1].

Iron deficiency anaemia (IDA) attributes to 55% of the global cases, and is frequently observed in young children (< 5 years), adolescent girls and pregnant women [1]. The World Health Organisation (WHO) categorised IDA in the top ten conditions contributing to the global burden of disease [1].

Anaemia is usually diagnosed using blood haemoglobin (Hb) levels. Hb is a protein molecule that resides in RBCs and has a main role to transport oxygen to body organs and tissues. Hb assessment can be performed by various methods such as Sahli's hemometer, which is one of the oldest methods that is still routinely used in developing countries [9]. In hospital settings haematology analysers are used, which provide detailed information on RBCs, haemoglobin and other blood parameters including white blood cells (WBC) and platelets. Recent advances include portable electronic devices such as the Hb-201 system (HemoCue, Sweden), which uses a finger prick blood sample, while the non-invasive technology NBM 200 (Orsense Ltd, Ness Ziona, Israel) measures haemoglobin without any blood withdrawal using infrared light technology [9,10].

In adults (male/female), WHO recommends a threshold of 12.0 grams/decilitre (g/dl) Hb to identify anaemia (anaemia if Hb < 12.0 g/dl) [2]. In pregnant women an 11.0 g/dl Hb cut off is recommended for the identification of anaemia. Clinically, anaemia is classified as mild, moderate and severe based on Hb levels. Contemporary management includes iron oral supplements in mild to moderate cases [2]. Severe anaemia on the other hand, may require hospital treatment to administer intra venous iron and correction of underlying pathological conditions [2].

1.3 Anaemia in India

India has the highest number of anaemic cases in the world [11,12]. Nearly 55% of pregnant women, and about 60% of adolescent girls are affected by the condition [13,14]. The prevalence varies within and across states because of change in dietary resources, healthcare access, and awareness of the disease itself [13].

A recent National Family Health Survey (NFHS 2005-06) reported that the prevalence was higher in rural areas as compared to urban regions [13]. In rural areas, government health services are the main providers of anaemia investigation and treatment; however, not all villages have government hospitals, and the majority are served by visiting nurses. These nurses are engaged in three major activities: implementation of vaccination initiative, national disease control programmes, and antenatal healthcare provision [15]. Currently, one nurse is assigned for every 7 to 10 villages (~10,000 individuals) [15]. As a result of the current rural healthcare structure and duties, nurses are burdened with routine activities while catering to a large population [16], which reduces opportunities to diagnose and treat existing anaemic cases. Along with the lack of workforce, medical resources such as equipment

supplies, medicine provisions, electricity and transport facilities are limited in rural areas. These factors in turn contribute to the high anaemia prevalence in rural regions compared to developed areas in the country because of under-detection and under-treatment [17,18].

1.4 PhD project background

This PhD project was a joint collaboration between the University of Nottingham, UK and Halo Medical Foundation, India [19]. The Maharashtra Anaemia Study (MAS) had three main components: adolescent girls survey, study of pregnant women with subsequent follow-up to ascertain maternal and birth outcomes, and the Maharashtra birth registry analysis.

A recent 2011 global anaemia report published by the WHO stated that adolescent girls and women of reproductive age group are at maximum risk of acquiring anaemia [1]. In rural Indian settings adolescent pregnancies and anaemia are common, which are clinically recognised as high-risk pregnancies. Schauberger described health issues related with adolescent pregnancies as follows [20]. Pregnancy at such an early age causes range of physical and psychological problems. For example, physical growth at young age is inadequate to ensure healthy pregnancy. Risks of premature birth, abortion, and psychological trauma are higher in such cases. Furthermore, risk of low birth weight, and poor neonatal outcomes are also higher in adolescents compared to older women. Data also demonstrate that adolescent pregnancy is likely to face serious complications such as intrauterine growth retardation, preeclampsia, and anaemia. Risk of mortality and morbidity is also higher in adolescents/young women than in older women.

Another important public health problem related with maternal health in India is sex selective abortion. Initial cases of sex selective abortions were detected in the late 1980s after the introduction of ultrasonography technology in the country [21,22]. Published reports indicated that more than 10 million female foetuses have been selectively aborted in the recent two decades mainly due to the importance given to male offspring in the societal and cultural set-up [21,22]. Although sex-specific foeticide is clearly fatal for the unborn female foetus, it also negatively impacts on female health. The chance of abortion increases if a female foetus is identified using ultrasonography technique. As summarised by Schauberger, adolescent pregnancies are high-risk cases considering various outlined reasons [20], which are likely to further increase the risk of complications following sex selective abortion. Abortion is a known risk factor for maternal anaemia and poor reproductive health outcomes [20]. Atrash and Houge conducted a review to investigate abortion and subsequent reproductive complications [24]. They described that abortion techniques have influence on pregnancy outcomes. For example, if dilation and evacuation method is performed for an induced abortion, then the risk of premature delivery and low birth weight is higher in subsequent pregnancies. Such early abortion in young girls/women is likely to increase risk of anaemia, due to blood loss and overall poor health status. Anaemia is a common health issue in rural India as outlined earlier, and repeated pregnancy termination (on sex identification) increases risks of infections, anaemia, and is likely to hamper future reproductive outcomes [20,24]. These issues increase an overall health burden during adolescent years, and also during pregnancy. Therefore, in Indian setting where early marriages are common, and also

a high prevalence of anaemia in women is documented, sex ratio investigation becomes an important public health inquiry.

1.5 Rationale

Published studies from India have documented a high prevalence of anaemia among the populations of adolescent girls and pregnant women. Some studies have also investigated anaemia risk factors; however there are many rural areas such as the Marathwada region of Maharashtra state, where data on this important public health issue are not available. Furthermore, research focusing on this unique population is limited due to poor infrastructure and development in this region. Information on anaemia is crucial for the future planning and practical implementation of much needed community health services focusing on adolescent and women's health. Additionally, such studies could also yield evidence that will be of use in other Indian states such as Rajasthan, West Bengal, Haryana and Punjab, where high anaemia prevalence in females is recorded.

Therefore, the primary goal of the PhD research (based on MAS) was to investigate adolescent and maternal health outcomes that are related with anaemia in Maharashtra state, India. MAS used WHO definitions outlined earlier in the introduction to identify and categorise anaemia in adolescent girls and pregnant women. The first objective was to investigate anaemia prevalence in both population groups, and study its risk factors. The second objective was to examine the association between maternal anaemia during pregnancy and adverse birth outcomes.

Female foeticide is a serious community problem, and is an important maternal health concern in the country as explained earlier. However data on female foeticide are very limited due to challenges involved in identification of such cases, and lack of an individual pregnancy monitoring system in India. Further obtaining data from abortion clinics or using self-reported data are not feasible options as conducting female foeticide is a criminal offence in the country. There is no published information available from Maharashtra state, and no data from government birth registries are presented from other regions of the country. Therefore, the third objective of the MAS project was to assess changes in sex ratio as a proxy for female foeticide which is carried out by abortion; abortion being a strong risk factor for anaemia, particularly in young women, reinforcing the cycle of subsequent poor health outcomes and adverse pregnancy outcomes, as described in the background. Specifically, the purpose was to assess whether the Maharashtra state sex ratio at birth has changed over the 32 years (1980 to 2011) as a potential marker of female foeticide.

Additionally, the MAS evaluated the application of non-invasive haemoglobin technology (known as NBM 200) in a rural Indian setting. Such evaluation is important considering poor health infrastructure in rural areas, where regular haemoglobin investigation is challenging as outlined in the introduction. There had been no previous field studies in India, nor in such rural areas, to assess this machine in the field, where its use is most practical and needed.

1.6 Goal and objectives

1.6.1 Study goal

MAS aimed to study adolescent and maternal health outcomes related with anaemia in Maharashtra state, India.

1.6.2 Primary objectives

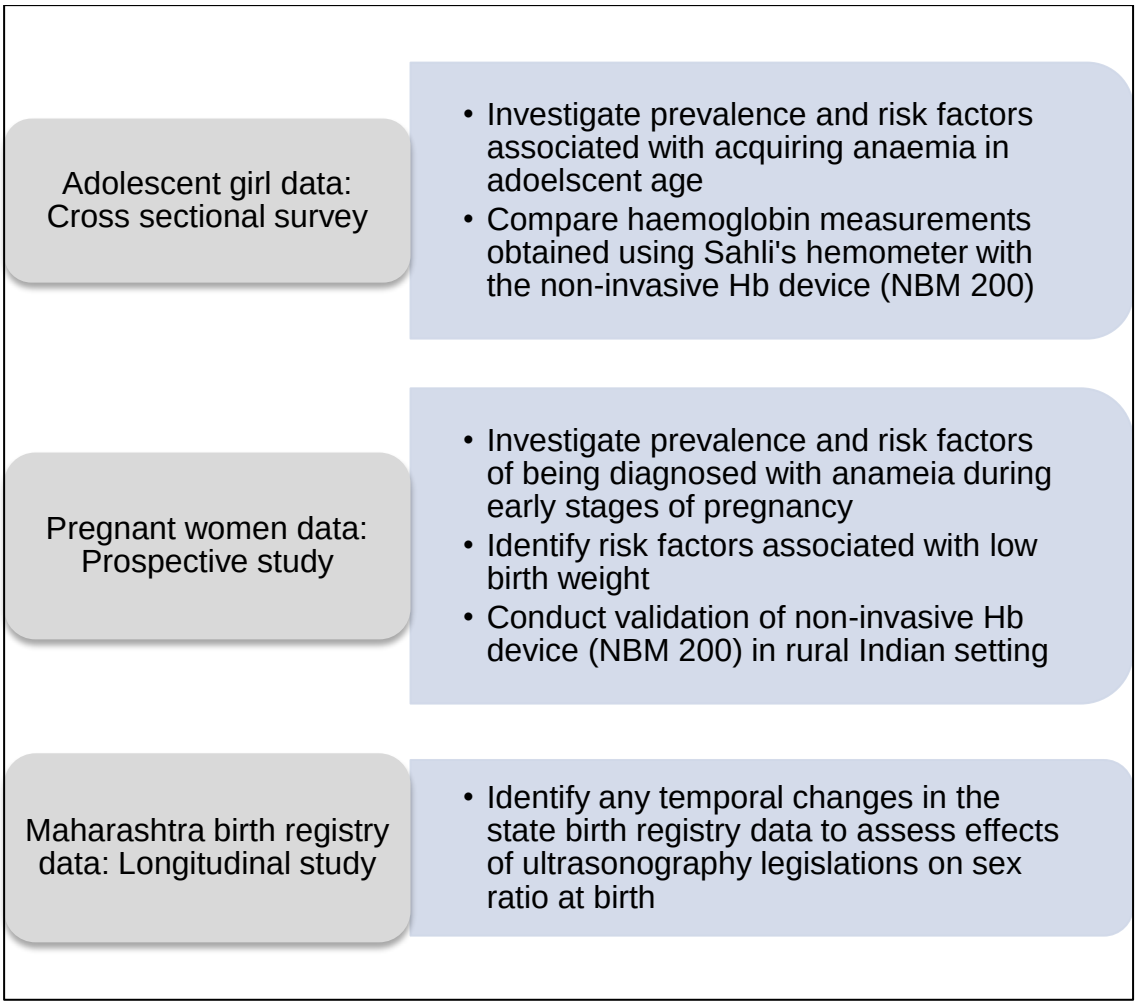
1. To estimate anaemia prevalence, and identify individual and village level risk factors associated with anaemia among adolescent girls (13 to 17 years) in Maharashtra.
2. To estimate anaemia prevalence, and identify individual and village level risk factors associated with anaemia among pregnant women in Maharashtra. The study also aimed to assess risk factors associated with low birth weight.
3. To study temporal changes in the sex ratio at birth of Maharashtra state over a period of thirty-two years (1980 to 2011) using anonymised routinely collected healthcare data to investigate effect of ultrasonography legislation.

1.6.3 Secondary objectives

1. To conduct comparison of the non-invasive haemoglobin technology (NBM 200) with Sahli's hemometer in adolescent girl population.
2. To validate the non-invasive haemoglobin technology of (NBM 200) using an automated cell counter (Sysmex XP-100) in rural pregnant women population.

A summary of the aims and objectives for each component of the project is presented in Figure 2.

Figure 2: Maharashtra anaemia study components with objectives



1.7 Thesis structure

The thesis has six chapters including the introduction (chapter 1). Chapter 2 outlines the general methodology, chapters 3, 4 and 5 report on the three main studies comprising this doctoral research. The last chapter summarises the thesis findings, and provides recommendations for public health practice and future research.
An overview of the next five chapters is given below.

1.7.1 Chapter 2. Methodology

This section provides detailed information on project development, study setting, study designs and ethical approvals. Information on the data collection process and quality controls are outlined in the chapter. Information on data analyses and additional details of each study component are in subsequent study-specific chapters (chapters 3 to 5).

1.7.2 Chapter 3. Adolescent girls: Cross sectional survey

The third chapter is focused on the adolescent girls survey where detailed information on study-specific methods is provided. The chapter reports the findings from the investigation of anaemia prevalence and risk factors associated with adolescent anaemia. Also the comparison of the non-invasive haemoglobin device (NBM 200) against the Sahli's hemometer Hb method is presented in the chapter.

1.7.3 Chapter 4. Pregnant women: Prospective study

This chapter reports on the pregnant women study findings on the prevalence of anaemia and risk factors associated with anaemia in pregnant women. The chapter

also discusses risk factors associated with low birth weight. The second section presents the validation study of the non-invasive haemoglobin device (NBM 200) against an automated haematology analyser in a rural Indian setting.

1.7.4 Chapter 5. Maharashtra birth registry: Longitudinal study

The fifth chapter focuses on the birth registry data from Maharashtra state of India. The analysis used 32 years of longitudinal data to investigate the impact of legislation banning sex determination on the state's sex ratio at birth.

1.7.5 Chapter 6. Conclusions and recommendations

The last chapter summarises the key thesis findings and discusses future research opportunities in India along with recommendations for public health interventions that could reduce the prevalence of anaemia at both local and regional level.

1.7.6 Appendices. Additional files

The appendices include the PhD project proposal, data collection forms, participant health report cards, ethics approval certificates, list of scholarship/bursary awards, standard operating procedures, and publications authored by the PhD candidate (Dr Anand Ahankari).

Chapter 2. Methodology

2.1 Introduction

This chapter describes the overall methodology of the Maharashtra Anaemia Study (MAS). The study setting, programme partners, ethical approvals and project management are described in detail. The second section of this chapter explains the data collection and quality controls. As outlined earlier in the introductory chapter, MAS had three main study components, (a) survey of adolescent girls, (b) pregnant women prospective study, and (c) analysis of Maharashtra birth registry data. This chapter provides the overview of data collection for each of these components. Additional details on the individual study-specific methodology and data analyses are mentioned in chapters 3, 4 and 5.

2.2 MAS setting

Maharashtra is the third largest state of India located on the west coast. The 2011 census recorded 109 million residents (2nd largest in the country) [25]. Mumbai is the capital of the state, and also serves as the financial capital of the country.

Geographically, Maharashtra has three major regions, Western Maharashtra, Marathwada and Eastern region. Though the state is recognised among the wealthiest states in India, most of the industrial and economic development is contained in Western Maharashtra, leaving the other two areas under developed. For example, the western region has a per capita annual income of 130,000 INR (1300 GBP), which is twofold that of the Marathwada region (67,000 INR, 670 GBP) [26].

Marathwada consists of eight districts, located across the southwest border of the state having a total population of 18.7 million with a regional literacy rate of 76% (Figure 3) [27].

Figure 2: Location of Marathwada region in Maharashtra state

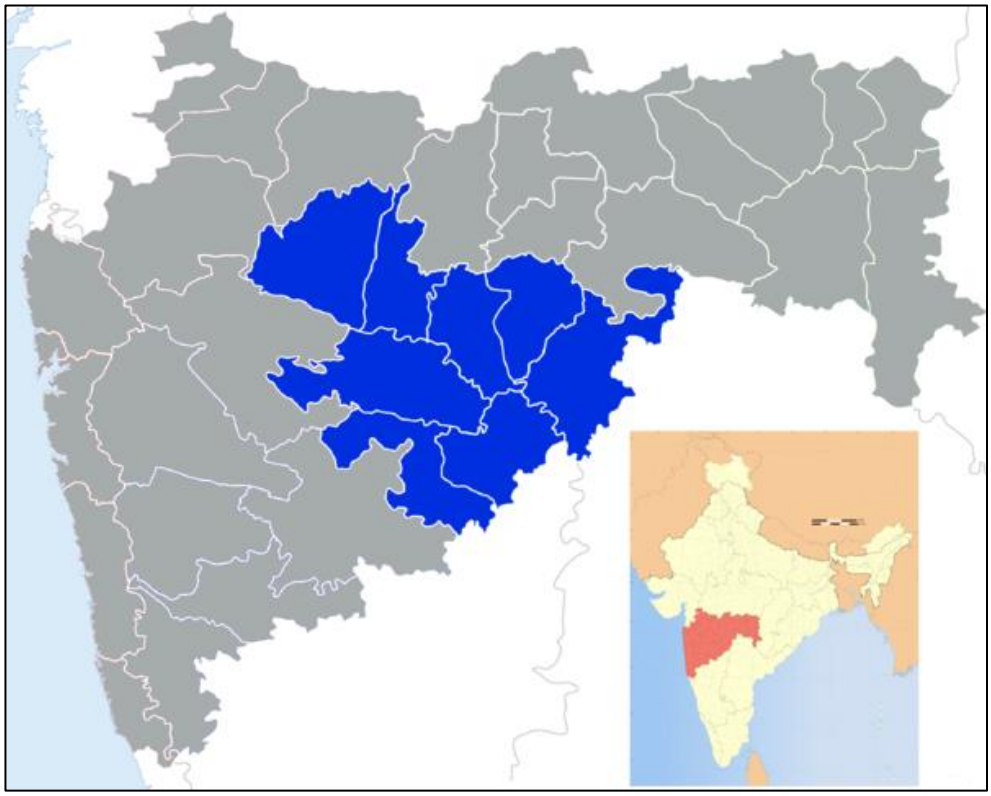


Figure 3 footnotes: Maharashtra is indicated in the right map (in orange colour) in India, and in the map on left, Marathwada region is indicated in the Maharashtra state (blue colour total 8 districts).

Source of figure: South Asia Network of Dams, Rivers and People (SAND).

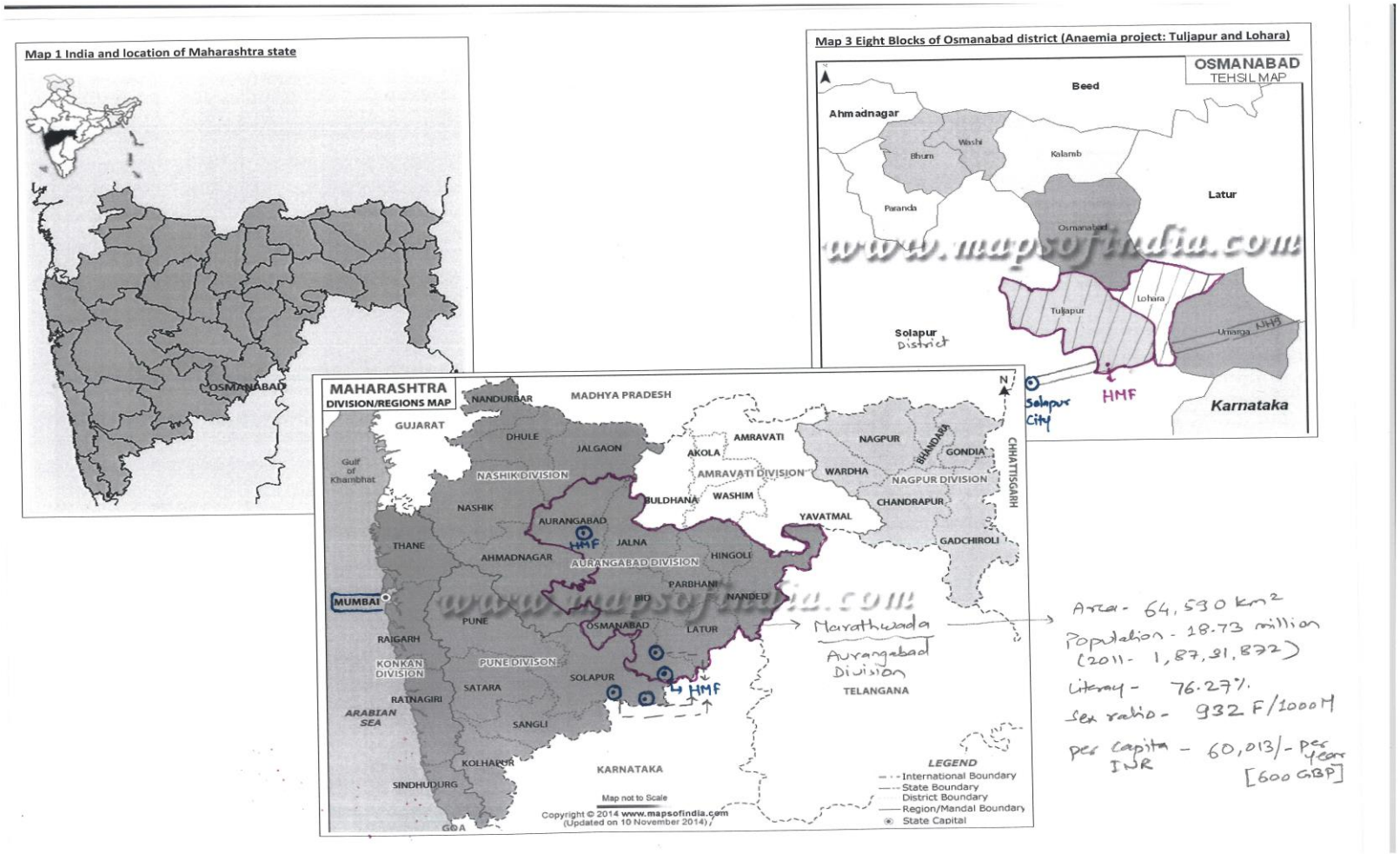
2.3 HMF and MAS

2.3.1 HMF background

The MAS was implemented in collaboration with Halo Medical Foundation (HMF), a non-government organisation (NGO) based in Marathwada region. HMF works to improve community health, economic situations of socially disadvantaged groups, and also provide health and science education to young people. The NGO has established healthcare services in rural Maharashtra catering a population of nearly 100,000 from 60 villages.

HMF's history: In the early 1990s, Osmanabad district from Marathwada (Figure 4) had an approximately 1 million population served by less than 100 medical doctors, with the majority of healthcare providers based at block or district cities [28]. The limited healthcare system was further depleted following the 1993 Killari-Latur Earthquake, which recorded more than 10,000 deaths and the displacement of about 100,000 people [29]. Following this disaster, HMF initiated the primary healthcare project (known as Village Health Workers) to establish a rural health workforce in the region in 1994.

Figure 4: Osmanabad District Map



Source of figure: Prepared by using various maps from Maps of India database.

Oxfam UK supported the first project of HMF, where a cadre of 30 female village healthcare workers (VHWs) was developed through a residential healthcare training programme. All these workers were women, permanent residents of Osmanabad district, and had completed a basic primary education enabling them to read and write in Marathi (language of Maharashtra state). On completion of the training VHWs were deployed to support the healthcare needs of their village, and were also connected with the HMF hospital to support their referrals. VHWs were trained to diagnose uncomplicated mild to moderate illness, and were provided with some 40 basic medicines and monthly remuneration, thus establishing basic primary healthcare services in the earthquake-affected villages. Workers were also trained to identify and refer emergency medical conditions such as snakebites, heart attacks and pregnancy complications, with the primary objective of identifying such cases at early stages. HMF expanded the village health workforce to 70 villages by 1996. During the first twelve years of the programme (1994 to 2006), VHWs provided consultations to nearly 110,000 patients in rural areas of Osmanabad district [28]. HMF also trained and established a similar healthcare workforce in three other Indian states (Madhya Pradesh, Gujarat and Karnataka). Following the success of the VHW model, the concept was used during the development of the ASHA programme (Accredited Social Health Activist) in the National Rural Health Mission of India (NRHM) [30]. Further in 2009, Maharashtra Medical University designed a one-year diploma course to train men and women to meet healthcare needs of the rural population using the VHW coursework. In addition to the VHW initiative, HMF has implemented more than 50 public health and development projects for women, young people and men with support from national and international donors from four continents. HMF also served as a mother NGO for government health projects, and

holds a key strategic position in Maharashtra state. Currently, HMF has an established network in 150 villages and 80 urban slums from Osmanabad and Sholapur districts (covering nearly 400,000 population). HMF's head office is based at Andur and has four main departments: two hospital units, a training centre and a science education department. Other than the head office, HMF has four block offices in field areas with a registered office at Aurangabad city, Maharashtra. At the time of writing this thesis, the trust employed 40 full time staff, 80 field workers and had 9 trustees.

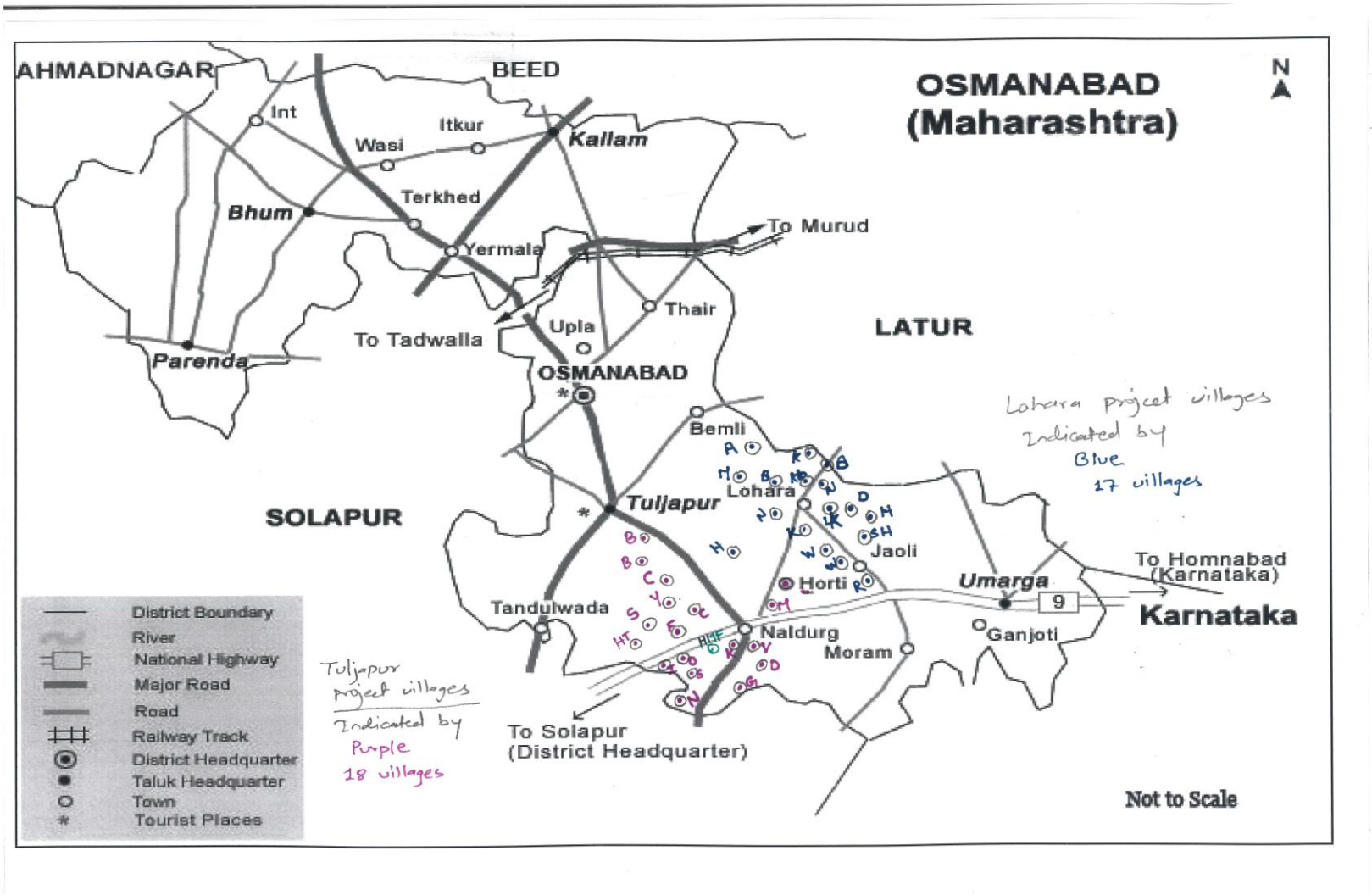
2.3.2 Maharashtra Anaemia Study (MAS)

The established network of VHWs and HMF's infrastructure were utilised for the PhD study. MAS was conducted in two blocks, namely Tuljapur and Lohara, situated in Osmanabad district of Marathwada where the HMF village health programme is in operation (Figure 5). The two blocks have 155 villages in total, having approximately 415,000 inhabitants [27].

Of these, MAS included 35 villages with a total target population of approximately 65,000 (Appendix 1 study village list). Four (of 35) villages were close to the National Highway 9 (NH 9- Mumbai to Hyderabad Road), while the rest were based in interior regions (> 10 to 15 kilometres from the highway) having limited transport and communication facilities. Being located in the Marathwada region, healthcare services and community infrastructure were inadequate. Electricity was available for an average of 14 hours a day, and tap water was provided every alternate day due to water shortage in Marathwada region [31]. These villages were linked with the HMF hospital located at Andur town, which provided both investigation and administration

support for the MAS project. The nearest village from HMF hospital was 5 kilometres and the farthest was 54 kilometres from the hospital. Photographs of the field area infrastructure and data collection activities are included in appendix 2. Additionally, a photo album was submitted with this thesis to the internal examiner (Professor Sarah Lewis, Division of Epidemiology and Public Health, University of Nottingham, UK).

Figure 5: MAS field area indicating villages from Tuljaur and Lohara blocks



Source of figure: Prepared by using a map from Maps of India database

2.4 MAS development and ethical approvals

The study was conceptualised during Dr Fogarty's visit to India (primary supervisor of the PhD project). The visit to the HMF head office took place in February 2013 to identify research opportunities in anaemia within the HMF's project area. During the visit, meetings with HMF secretary and staff were facilitated by Dr Ahankari (PhD candidate). Field assessments were made to understand research scope and feasibility. In total, 3 villages were visited from HMF's field area.

Initially a concept note was developed in March 2013, and the full proposal development was started in June 2013 with PhD supervisors (based at the University of Nottingham, UK). The draft was finalised by July 2013 following inputs from the HMF review board (Appendix 3 PhD project proposal). The internal approval for the MAS project was obtained from the HMF board of trustees in August 2013. The ethics application was initially submitted to the Government Medical College of Aurangabad, Maharashtra, India. The Medical College committee was the only authorised ethics body in the Marathwada region in 2013 (Registration number: ECR/314/Inst/MH/2013, authorised by the Home Ministry of India, New Delhi). As a primary requirement of the committee, a co-investigator (Dr Jagannath Dixit) was appointed for the project from the Preventive Social Medicine Department of the Medical College. Dr Dixit's role in the project was to provide local support whenever needed, and act as a liaison with the Indian ethics committee based at Aurangabad. Dr Anand Ahankari (PhD student) was registered as the Primary Investigator (PI) for the MAS with the Indian ethics committee, which was an additional requirement for the application. Approval from the Indian ethics committee was received on 25th

September 2013, following a presentation by Dr Dixit at the Medical School, Aurangabad.

The UK ethics application was submitted to the Nottingham Medical School Ethics Committee in October 2013, and was sanctioned on 1st November 2013. Ethics approval certificates are provided in appendix 4 (Ethical approvals).

Ethical approvals

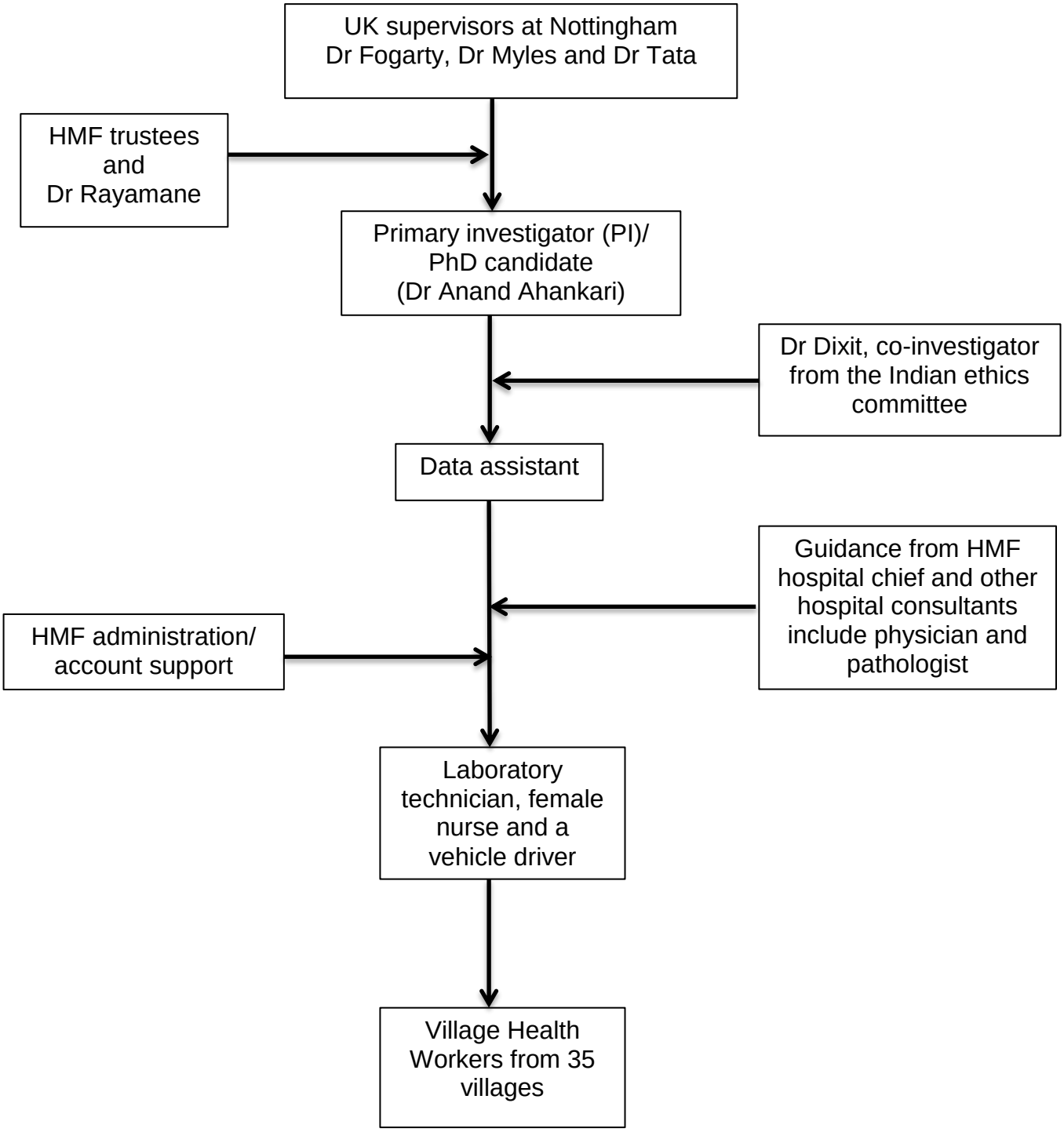
- Institutional Ethics Committee of Government Medical College Aurangabad, Maharashtra, India (Reference number: Pharma/IEC-GMCA/331/2013).
- University of Nottingham (UK) Medical School Ethics Committee, United Kingdom (Reference number: E10102013).

MAS received an additional grant from the University of Nottingham, UK to include NBM 200 technology assessment in February 2014. The grant amount was used to purchase the device. Therefore, the initial ethical approvals were updated to involve NBM 200 assessment in the project. The revised ethical clearance was granted in March 2014 from the Nottingham University, and was further cleared from the Indian ethics board in April 2014.

2.5 Project management

HMF was the implementing agency for the MAS. A project grant was obtained through an individual donation from Dr Sulbha Hardikar (based at Texas, USA), who provided the financial support directed by the Maharashtra Foundation, New York, USA (award amount: £17,500) [32]. Other individuals and organisations who provided financial support for the MAS project and PhD studies are outlined in appendix 5 (Scholarships and Grants). MAS had a separate office space with dedicated non-medical and medical staff engaged on a full time basis. HMF also provided access to its administration team for accounting and general support purposes. The project was launched in December 2013, following the appointment of Dr Ahankari (PhD candidate) as a Research Project Coordinator/Primary Investigator (PI) (full time appointment in India, December 2013 to June 2015). The PhD candidate was trained in Dentistry in India, and had 2 years of full-time working experience prior to this appointment [with HMF, India (for 18 months), and with the University of Nottingham, UK (over 6 months)]. Dr Ahankari's main responsibility was to implement the study according to the protocol, and also participate in HMF's initiatives related to the MAS. Four other support staff members were appointed for the project: a data collection assistant, female nurse, laboratory technician and a vehicle driver. All project staff were employed on full time basis, and appointed for the same duration as the PI. HMF also assigned Dr Kranti Rayamane, (Secretary of HMF) a monitoring role involving a monthly teleconference with Dr Ahankari. Additional support was provided by the HMF review board, which included three members from the HMF board of trustees and two advisors. MAS organogram is outlined below (Figure 6).

Figure 6: Organogram of Maharashtra Anaemia Study (MAS)



2.6 Data collection forms design and pilot process

Three data collection forms were developed for obtaining adolescent girl data, pregnant women data and village profile data. All three forms were prepared at Nottingham during the first four months of the PhD period (September 2013 to December 2013). Forms were initially designed in the English language, and were revised following series of discussions with supervisors. Data collection forms were also shared in November 2013 with HMF, where Dr Rayamane facilitated a discussion with HMF staff, trustees, advisors and village health workers. The meeting provided opportunity for HMF to provide feedback on data forms based on their experience and understanding of the study area. No major changes were suggested in the meeting. The data forms were agreed on 15th December 2013 (English version).

English language data forms were translated into Marathi language (local language) by the PI (PhD candidate, bilingual proficiency). A HMF review board member, who also had a bilingual proficiency, translated all three forms independently. No major differences were seen in the translation performed by the two members. Data forms were piloted between January to February 2014 with six adolescent girls, and two pregnant women. The village level data profile form was also piloted in four villages. No changes were deemed necessary in the village level data format. While in the other two forms, four minor changes associated with multiple choice questions were made.

Back-translation: Approved copies of all three data collection forms (in Marathi Language) were submitted to the Trans Solutions Agency based at Pune city,

Maharashtra in February 2014. The agency back-translated all three forms in English independently, and those were shared electronically with the PI and UK supervisors at the same time. Back-translated English forms were assessed by the PI and PhD supervisors, and were found satisfactory. Final versions of data collection forms are included in appendix 6.

2.7 Staff training and pilot process

HMF's village health workers underpinned the MAS data collection. Each village had one female healthcare worker, and thus, a total of 35 workers with an average working experience of 5 years contributed to the study. All healthcare workers were permanent residents of their own village, and were appointed to work on the study for three days a month during the project period. A monthly remuneration was provided for the three days of contribution. Of the three days, one day was assigned for a monthly meeting at HMF office, and two days were dedicated for house surveys/data collection support duties.

In total two staff trainings were conducted for MAS staff in local language (Marathi). Each lasted for 6 hours, and both were conducted at the HMF office by the PI.

2.7.1 Training 1

VHWs participated in the first training in January 2014 where they were informed about the project purpose, objectives and overall data collection process. Staff members (data assistant, nurse and laboratory technician) were also involved in the training. Through the first training special emphasis was given on individual conduct in the study, the participant's right to reject study involvement, the importance of

voluntary participation in research programmes, ethical concerns in international research, data security and confidentiality in research projects. Information on anaemia during adolescence and pregnancy was also discussed. The training involved mock data collection sessions, which were supervised by the PI, and then further feedback was obtained from HMF review board members who participated in the training. Dr Laila Tata (PhD co-supervisor) visited HMF in January 2014, after completion of the first staff training. Research office facilities, data storage protocol, resources and pilot process were reviewed by Dr Tata, and were found satisfactory in accordance with the MAS Standard Operating Procedures (Appendix 8 SOP).

2.7.2 Training 2

The second staff training was conducted in March 2014, and it was focused on the project management and individual responsibility as well as MAS timelines.

2.8 Data Collection

2.8.1 Adolescent girl data collection: Cross sectional study

Data were collected from adolescent girls to estimate the prevalence and risk factors of anaemia in a cross sectional survey (see appendix 6 for data form).

Eligibility and data collection process

All unmarried adolescent girls from 35 project villages between 13 to 17 years were eligible to participate in the study. We decided on this age-group following consultation with HMF. The upper age limit (17 years) was suggested because of early marriages (typically at the age of 18 years), and also because of the migration

of girls for higher secondary education in our study region. The lower age limit (13 years) was agreed following ethical requirements where an ability to make an independent decision was necessary to provide written consent to participate in the study. As a primary step, a list of adolescent girls from 35 villages was prepared from December 2013 to March 2014. The identification was done through house surveys by VHWs, who were provided with necessary tools and support at village level. The data assistant checked eligible lists with local government offices and school administration, wherever required. In total, 1,898 eligible girls were identified through this process. The study aimed to collect data from the first 1000 participants. No formal sample size calculations were performed, as the MAS project was a feasibility study to identify future research priorities in Maharashtra.

Field visit planning

Village visits were planned during monthly meetings with VHWs. Each visit was scheduled in coordination with staff members, and then necessary arrangements were made such as booking an HMF vehicle for the data collection day. The data assistant and the PI were responsible for planning the field visit with additional support from VHWs. Data collection was mainly performed on weekends/public holidays at village health centre or other suitable sites accessible from all areas of a village (example- school, village health centre). Special care was taken while selecting a place such as clean seating area, ample daylight, secure rooftop, tiled floor as these factors were deemed necessary for safety of both, project staff and study participants. During each visit, efforts were made to place measurement equipment according to guidelines, such as placing the weighing machine on a flat floor. During the village setup, equipment accuracy was checked (such as weighing

machine's performance) by both the assistant and the PI prior to the start of data collection session.

Once the site was prepared, VHWs visited eligible girls in the village on the data collection day to invite participation in the study. Girls and their parents/local guardian reported at the data collection site, following which the PI or data assistant briefed eligible participants about the study. Each adolescent girl provided a written consent that was counter signed by their parent/local guardian. No monetary remuneration was given for participation in the study. Those who agreed to participate were initially administered a questionnaire (recorded by data assistant or the PI), and later were referred to a nurse who recorded all measurements (such as height, weight, mid upper arm circumference), following which the participant had the haemoglobin investigation. Haemoglobin was measured at village level using Sahli's hemometer technique, and results were obtained immediately by the study technician in the field. Additional details on the hemometer technique are described in the Appendix 8.
As a secondary objective of the study, haemoglobin was also recorded using a non-invasive device NBM 200 to facilitate comparison of measurements obtained using the two methods. Details of the NBM 200 technology are described in Chapter 3 (page number 67).

On completion of all tasks, each participant received counselling by the PI where their health report card and Hb value were explained, and information on anaemia prevention was also provided. The report card had necessary details on how to access HMF's village health services along with contact details of HMF hospital and the PI (Appendix 7). For each participant 20 minutes were required to complete all

three stations. Those who declined to report to the data collection centre, and also those who were rejected to participate after the study brief were recorded in a separate register by the PI, and were not approached a second time by any means across the study tenure. All efforts were made to reduce any peer or parental influence to allow each participant to make their own decision. All data collection, physical measurements, staff conduct and referrals were made according to the Standard Operating Procedures developed for the MAS (Appendix 8). During the consent process and across all data collection visits, the PI was present at the site across the project period. Those identified with severe anaemia were noted in a separate register, and the accompanying person (parent/local guardian) also received counselling to ensure that a further consultation with local doctor/nurse/nearest hospital was arranged as a matter of urgency. HMF hospital was made available with free access to consultation, investigation and supplementation treatment. No field visits were made in the absence of the PI. All services including data collection, investigations, referrals and post-recruitment consultations at HMF hospital were free to all study participants.

Villages were prioritised by population size (ascending order) for data collection visits. Adolescent data collection was performed in two phases. During the phase 1 (summer vacation), data were collected from seven villages between 24th April 2014 and 10th June 2015 (209 participants, one visit per village). The second phase was planned from 10th January 2015 to 2nd May 2015. During the second phase, data were collected from 814 girls from 27 villages. Villages with more than 2000 population were visited twice to approach all eligible participants. Field visits were completed by May 2015. In total 51 village data collection visits were made for

adolescent groups. Being a cross sectional study, participants were contacted only once during the study period.

2.8.2 Pregnant women data collection: Prospective study

Data from pregnant women were collected to investigate the prevalence and risk factors of anaemia during pregnancy, and participants were followed to record pregnancy outcomes (primary outcome of interest- birth weight) (see appendix 6 for data form).

Eligibility and data collection process

Pregnant women who were residents of the 35 study villages, and between the 3rd and 5th month of pregnancy in the period 24th April 2014 and 31st December 2014 were eligible to participate in the study. The government health services in the field areas advise registration of pregnancy by 12 weeks to engage women in national vaccination and supplementation initiatives. Based on HMF's experience, the earliest identification of pregnant women was feasible by 8 weeks, and thus we decided to include pregnant women from the start of 3 months (from 8 weeks) of pregnancy until completion of 5th month (20th week). The upper limit (5th month) was agreed to take into account the local custom where pregnant women travel to live in their mother's/parent's house generally before the start of the third trimester. Subsequently, the baseline recruitment of all eligible pregnancies from a fixed pregnancy period (3 to 5 months) was agreed to develop a uniform cohort to compare pregnancy outcomes following post-delivery data collection. Women were recruited in the fixed timeframe (April to December 2014) to enable collection of post-

delivery data within the permitted timeframe according to the Indian Ethics Approval. The post-delivery data collection was completed by 30th June 2015.

Field visit planning

Village health workers (VHWs) were assigned to conduct house surveys in their own village (once a month), where women in the reproductive age group (15 to 49 years) were visited over an 8-month period (April 2014 to December 2014). Those who reported missed menses were advised by VHWs to conduct pregnancy test. VHWs were also provided with pregnancy test kits to conduct free investigation at village level. Identification of any new pregnancy was reported in the monthly staff meeting, which were recorded by a data assistant. Eligible lists of pregnant women were maintained through this process. Additional verification was also performed on bi-monthly basis with government nurses working in the study area to ensure that none of the eligible pregnancies were missed. No formal sample size calculations were performed. Based on HMF's maternal health programme in Osmanabad district about 5 pregnancies were expected every 1000 population every six months, thus in total a maximum of 325 pregnancies were expected during the recruitment duration (April 2014 to December 2014) [33]. In order to reach all eligible pregnancies in the project area in the given timeframe, pregnancy data collection was initiated on priority from day one- 24th April 2014. About 15 villages were visited twice and other mainly large villages (>2000 population) were visited four times to complete the recruitment. The pregnant women recruitment was completed by 31st December 2014. In total 76 village visits were made to collect data from pregnant women.

Village visits were planned during monthly meetings with VHWs following listing of eligible pregnant women. A data assistant maintained communications with VHWs over the telephone to record necessary updates. Once the data collection day for the village was confirmed, VHWs informed eligible women, and requested willing participants to be in the village on the assigned day. For pregnant women, data collection was performed at the participant's house or at the village health centre according to their convenience. Considering local cultural norms, no visits were made on religious days or festivals. Visits were made on weekdays and weekends as advised by VHWs.

Initially the data collection site was prepared as outlined earlier. Eligible participants were informed about study objectives, types of questions in the questionnaire, blood withdrawal, reporting system and post-delivery data collection plan. Along with consent from the eligible woman, additional written permission was also obtained from the participant's spouse/in-laws/family member. Each eligible participant was approached only once for the recruitment, and reasons for refusal were recorded in those who declined participation. For pregnant women, venous blood withdrawal was performed at village level, and the same was transported to HMF hospital to conduct blood investigations (Complete blood count and Serum glucose tests). Details of blood withdrawal and investigations are described in the chapter 4. Haemoglobin was also recorded using a non-invasive device (NBM 200) to evaluate the device performance in rural Indian pregnant women. On completion of blood investigations at HMF hospital participants were informed of the results over a phone call by the PI, which followed necessary referrals. Those with severe anaemia received printed reports within 24 hours of recruitment, and were referred to their chosen hospital.

Others with mild to moderate anaemia received reports within 72 hours from the recruitment, and were advised to visit HMF hospital/ nearest healthcare facility for further medical consultation.

Data collection, physical measurements, study conduct, blood withdrawal, referrals were made according to the Standard Operating Procedures developed for the MAS (Appendix 8). All efforts were made to reduce any influence from in-laws and spouse by providing sufficient time to each participant to make an individual decision. During all data collection visits, the PI was present at the site. No field visits or blood withdrawal were made in absence of the PI. All services including data collection, investigations, referrals and consultations at HMF hospital were made freely available to all study participants.

Post-delivery data collection

During the data collection mobile contacts of the participants/their spouse/their family member were recorded for follow-up. All participants were contacted by telephone two months after pre-delivery data collection to enquire about their overall wellbeing. Any changes in the expected date of delivery (EDD) were recorded for those who had undergone ultrasonography investigations in preceding two months. A second follow-up phone call was made by the data assistant in the third trimester, and changes in the EDD, and pregnancy status were recorded. Post-delivery data were collected through a third telephone call to the participants within one week of delivery. Data were collected on the number of hospital visits during pregnancy, any disease/condition diagnosed after the baseline recruitment, blood transfusion, delivery location (hospital/home), delivery type (normal/assisted/caesarean), birth

weight, and information on maternal/neonatal complications (if occurred any). Birth weight from all in-hospital deliveries were recorded based on their post-natal health records (hospital discharge card). For those who had home deliveries, birth weight was recorded within the first two hours of birth, at nearest health centre by a qualified healthcare provider. VHWs confirmed post-delivery health records whenever required. For example, if the participant reported birth weight more than 3.5 Kg over telephone, VHW checked the hospital discharge card to ensure accuracy of the information. Post-delivery data collection was completed by 30th June 2015. VHWs followed each participant until 42nd day from the delivery to record any delayed pregnancy outcomes, and were recorded if occurred.

2.8.3 Village profile data

Village profiles were prepared to identify risk factors associated with anaemia during adolescence and pregnancy. Data collected included information on village governance structure, number of women in the local government body, village population, health and education committees, water provisions, transportation and communication (see appendix 6 for data form). Healthcare information included details on government nursing provision, number of visits per month, availability of haemoglobin investigation and blood pressure measurement, access to private doctors, ambulance service and medicine supplies.

All government services are available at no cost in the study area, while private healthcare is a paid facility. Village level data were obtained initially from VHWs, and later confirmed with government offices wherever necessary. Data were collected only once in each village between February and April 2014.

2.8.4 Birth registry data collection

Birth registry data were collected to study changes in the state sex ratio at birth to investigate impact of ultrasonography legislations (introduced in 1994 and in 2003). Through the HMF's existing network with the government health department, a meeting was organised with the District Health Officer (DHO) and government statisticians. Two meetings were conducted at Sholapur and Osmanabad district offices in July and August 2014. During the meeting, government protocols for recording new births were discussed, and the PI was advised to contact the Health Department based at Pune city, Maharashtra.

The Health Department located at Pune was contacted in August 2014. An appointment was secured with the secretary, where the PI presented the PhD project for 15 minutes, focusing mainly on the birth registry data collection. Approval was obtained from the director, and the PI was assigned to work with the health record division at Pune. The record division maintained manual entries, which were used to prepare annual state health reports. The state health report had summary statistics on total births and deaths across Maharashtra state. The information was reported in total numbers for each district over 32 years of period (1980 to 2011). All annual reports were stored as printed hard copies. Information on annual births from year 1980 to year 2011 were extracted from each report by the PI, and later data were entered in computer at HMF research facility by a data assistant. The PI verified all birth registry data entries with the originally extracted data sheets. Being a retrospective longitudinal analysis, the Health Department was contacted only once and birth registry data collection was completed by October 2014.

2.9 Data characteristics

2.9.1 Building the dataset and security

Adolescent and pregnant women data: Data were collected on pre-printed forms at village level. The forms were used to prepare a dataset in Microsoft Excel at the research office located at HMF head office in Andur. The research office was a separate unit based on the first floor of HMF hospital with access only to the PI, and a data assistant. The office had safe cabinets (key access) to store data forms, and the keys were stored with the PI at all times. None of the other members of HMF including review board, trustees, staff members had an independent access to the research office.

A data assistant initially entered each data collection form. In total 1023 adolescent girls records, and 296 pregnant women forms were entered in the computer. The PI checked all entries for accuracy, and corrections were made wherever required. Each participant had a unique identification number (Participant ID), with no personal information such as name, address, phone number recorded in the computer at any time.

The state birth registry data were available in summary statistics with no individual information available/obtained. A data assistant entered all birth registry data in the computer. The PI checked each entry, and no discrepancies were observed. Monthly backup files of electronically entered data at HMF research office were stored on the Nottingham Research Drive through remote Internet access. The research drive had a secure folder for the MAS project (known as: HaloData) providing access to four members, the PI and three supervisors based at

Nottingham. All documents included study progress reports, equipment report, anonymised dataset were stored and updated on a monthly basis by the PI. No data were shared with any Indian or UK team member via emails or any other method of communications.

Project progress, conduct and data security were assessed during a visit made by the chief supervisor (Dr Fogarty) in October 2014. In total three visits were made by UK supervisors; first visit by Dr Fogarty during project development (February 2013), second visit by Dr Tata after establishing the research facility (January 2014), and the third visit by Dr Fogarty after the first six months of data collection (October 2014). Dr Rayamane visited the research office twice to ensure the quality and research conduct (June 2014 and January 2015). Dr Dixit visited in February 2015 to observe project progress and study conduct, and the same was reported to the ethics committee in India and UK.

2.9.2 Quality controls

All study tools such as weight, height, blood pressure apparatus and mid arm measuring tapes were checked on the 1st working day of each month. The comparison was made with the same set of instruments stored at the MAS research office. A monthly equipment report was generated and shared with the UK team. No faults were detected with any study equipment other than the non-invasive Hb device (NBM 200). The NBM 200 unit had technical problems twice during the study period, and was replaced by a new unit from the authorised supplier (Span HealthCare Pvt Ltd, India).

Hospital laboratory machines included a cell counter (to measure complete blood count), and a biochemistry analyser (to measure serum glucose). Both were calibrated everyday as per the manufacturer's protocol. During the study period, both machines were checked for quality performance, every three months by a service engineer. No major repair or services were required during the study period.

MAS study progress reports were shared every two weeks in the first two months of data collection (April to June 2014), and later reports were shared on a quarterly basis with the UK supervisors and HMF review board.

When the PI was based in India, monthly meetings were conducted with the UK team to ensure continuous monitoring during the study period (3 meetings over Skype, 15 through email communications and 3 in person during Dr Tata and Dr Fogarty visits to HMF). Minutes of all meetings conducted over PhD period were documented and submitted to the university (total 96 meetings in 42 months).

2.9.3 Responsibility towards study participants

All study participants (adolescent girls and pregnant women) received an investigation report following their participation in the study. VHWs provided village education and support services to study participants. Special facilitated access to HMF's hospital or a government health centre was also provided depending on the participant's choice. Severe anaemia cases were urgently referred to nearby hospitals. For adolescent participants, before advising any referral, parents or local guardians were consulted. All report cards had contact details of the PI with continuous access over telephone across project duration.

Through the MAS, prior to the start of data collection activities, six adolescent gatherings were conducted in two blocks that were attended by 823 adolescent girls in total. At these gatherings, information on anaemia, causes, disease prevention and ways to access health facility, was provided by the PI. On completion of data collection, VHWs were provided with an additional reminder with details of severe anaemic cases to ensure that necessary consultations were obtained. The MAS data collection phase was officially closed on 4th July 2015, and all data forms, office documents, administration record and soft copies were transferred to the central HMF data storage facility, where they will be stored for seven years in accordance with ethical guidelines and regulations.

2.10 MAS study designs summary

2.10.1 Adolescent girl cross sectional study

Data were collected from adolescent girls through a cross sectional survey conducted between 24th April 2014 and 2nd May 2015 to identify prevalence and individual risk factors associated with anaemia. Haemoglobin investigation was conducted at village level using Sahli's hemometer technique and NBM 200 device, and counselling was provided to all study participants. Participants were approached only once, and those with severe anaemia were urgently referred. Other participants with mild to moderate anaemia were referred to local health facility.

2.10.2 Pregnant women prospective study

Data were collected from 3 to 5 months of pregnant women identified between 24th April and 31st December 2014 to identify prevalence and risk factors associated with

anaemia. Venous blood withdrawal was performed at village level, and blood samples were transported to the HMF hospital. Hb was also measured using the NBM 200 device. Complete blood count and serum glucose values were investigated, and all participants received reports in 72 hours of withdrawal. Those with severe anaemia were referred within the first 24 hours of recruitment. Participants were contacted over telephone after seven days of delivery to collect data on pregnancy outcomes. All recruited participants were tracked and followed up by VHWs since the day of recruitment until 42nd day post-delivery.

2.10.3 Village level data collection

Data on study villages were collected to identify risk factors associated with anaemia. Village profiles were prepared for each village with information on governance structure, health facilities, water supplies, transport facilities and communication resources. Information was collected only once from February to April 2014, and the same was verified with the village governance office and government nurse wherever necessary.

2.10.4 Maharashtra birth registry longitudinal study

Maharashtra state birth registry was used to collect data on 32 years of births (1980 to 2011). Data were collected only once from the Health Department based at Pune city, Maharashtra to study temporal changes in the state sex ratio at birth to investigate effects of ultrasonography legislations.

Chapter 3. Adolescent girls: Cross sectional survey

3.1 Introduction

This chapter is focused on the first component of the MAS (Adolescent Girls Survey). It has four sections namely literature review, methods, results and discussion. In the literature review, published studies on anaemia from India including those from Maharashtra state are summarised. The second part of the literature review explains the current evidence gap, and purpose of this survey. In the methods, the survey objectives, assessment of blood haemoglobin levels, and statistical analysis are described. The results comprise of individual and village level risk factors of anaemia, and the second part reports on the non-invasive haemoglobin technology assessment (NBM 200). Discussion has summary findings of the survey followed by synthesis. Manuscripts developed from this chapter are listed in the appendix 12 (list of publications).

3.2 Literature review

Iron deficiency anaemia (IDA) is a common condition observed in Indian adolescent girls [13]. About 60% of girls in the country aged 10 to 19 years are affected by IDA [13]. The condition may impede physical development, which may continue to influence reproductive health outcomes [34]. The Indian government has emphasised the need of both, education programmes and national iron supplementation initiative. However, actual the implementation process has revealed important healthcare challenges such as lack of human resource particularly in rural areas, poor

monitoring, and inadequate iron tablet supplies [18,35]. As a result IDA prevalence has remained much higher than the predicted declines [18,35] .

3.2.1 Published findings from India and Maharashtra state

The anaemia prevalence varies within Indian states, and also by region; Southern, central and northern regional prevalence varies approximately by 20% [36].

A national survey conducted in 2009 involving 35 Indian states reported an overall national prevalence of 89% with substantial regional variation (Figure 7) [36]. This variation is likely due to development, healthcare facilities and food resources, which differ extensively within Indian states. Maharashtra state as indicated in figure 7 is among the five states where high prevalence is recorded (>80%).

The lowest prevalence in south India was seen in Kerala state (about 25%) [36], while studies from Tamil Nadu reported a prevalence between 40% to 55% [37–39]. Other states such as Karnataka and Andhra Pradesh have more than 50% prevalence [36,40]. In northern India, Himachal Pradesh (a state located north to New Delhi) reported the lowest prevalence (about 27%) compared to neighbouring states such as Haryana, Punjab, Rajasthan, Uttar Pradesh and Bihar where high prevalence was seen (75% to 95%) [36,41–45]. Similarly eastern states include West Bengal and Orissa recorded a high prevalence (more than 80%) [46,47].

Figure 7: Anaemia prevalence across Indian states (prevalence increases with the colour intensity).

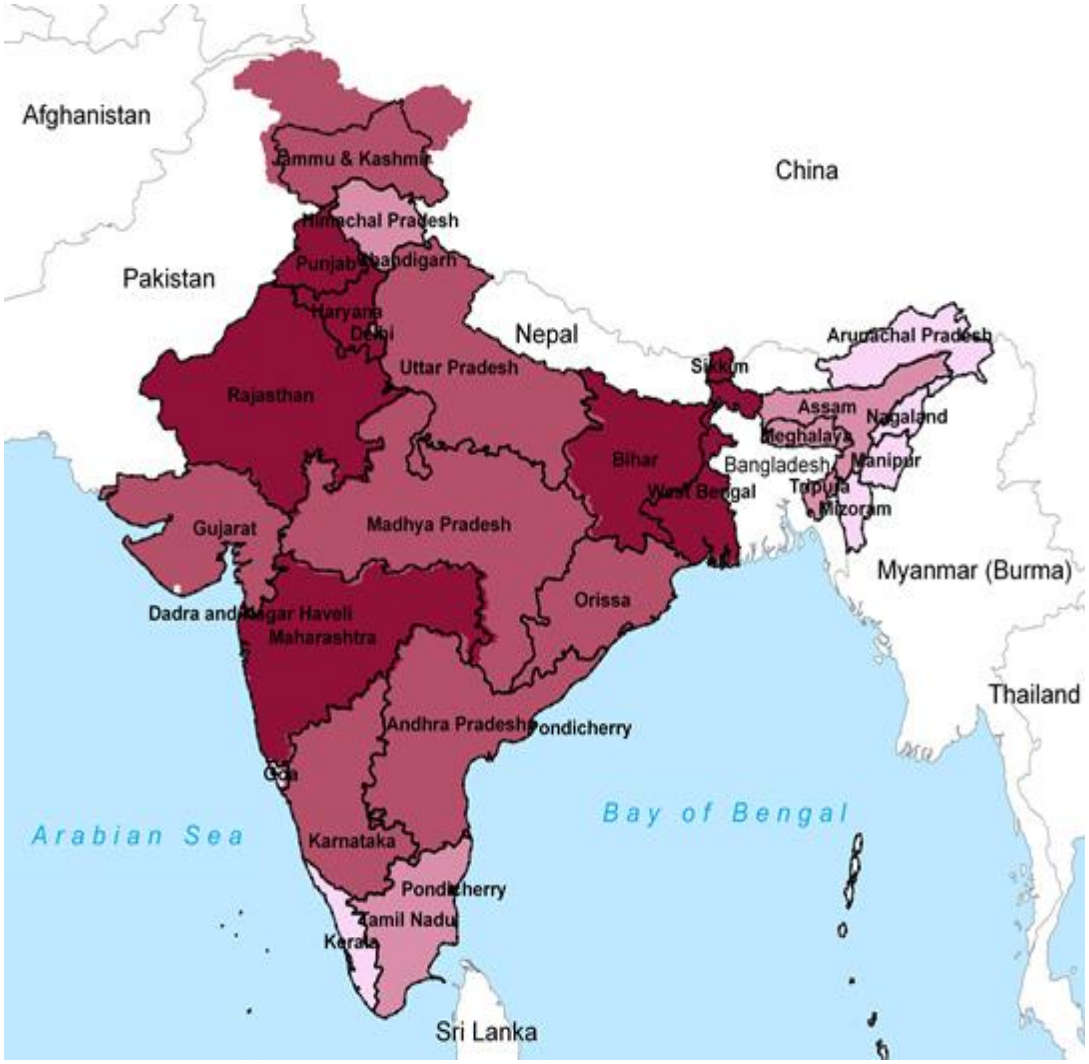


Figure 7 footnotes: Highest prevalence is seen five states include Haryana, Punjab, Bihar, West Bengal and Maharashtra. Lowest prevalence was seen in Himachal Pradesh, Arunachal Pradesh, Manipur, Nagaland, Mizoram, and Kerala.

Figure source: Bharati et al. Burden of anemia and its socioeconomic determinants among adolescent girls in India. *Food Nutr Bull*. 2009;30(3):217-226. [36]

Several studies were conducted in India to investigate the prevalence as summarised above, however very little is known about the risk factors of anaemia in the adolescent female population. Studies on risk factors of anaemia were searched using PubMed, EMBASE and Web of Science databases. Only two papers were found where detailed risk factor analyses were performed using regression techniques. However in the other 29 papers merely the anaemia prevalence is stipulated (Table 1). The national survey covering 35 states outlined earlier is one of the two papers which reported risk factors, but the investigation was limited to only four variables namely area of residence (urban/rural), caste/religion, education and age [36]. The second study assessed five factors namely Age, BMI, Mid Upper Arm Circumference (MUAC), education and family income [47]. To our knowledge, no detailed investigation is published from an epidemiological perspective looking at individual and village level risk factors of anaemia in the country.

Studies published from Maharashtra state reported 40% to 65% of prevalence in the western region [48,49], and 35% to 40% in the eastern region [50]. Higher prevalence was observed in deprived and rural areas (up to 90%) [51], which is likely due to limited healthcare facilities, poor development, and lack of awareness compared to urban or semi-urban areas [36]. Evidence from central Maharashtra (the Marathwada region) is limited to a 2013 study of 387 adolescent girls (from semi-urban area), which reported 68% anaemia [52] (Table 2). There are no published studies from rural areas particularly in the Marathwada region of Maharashtra state on anaemia prevalence in adolescent girls, and overall there is limited evidence at national level about risk factors associated with anaemia as outlined above.

Table 1: Published studies on anaemia involving adolescent girl population in India (17 studies)

Author, publication year and study type	Study location in India (setting and state)	Sample size and age group	Risk factors studied	Anaemia prevalence in adolescent girls unless indicated differently	Additional information/analysis
Goyle et al 2009 [53] Survey	Urban area, Rajasthan state	148 (10 to 16 years, girls) Ten schools from Jaipur city	None	96%	Prevalence of anaemia and serum iron levels
Bhardwaj et al 2013 [42] Survey	Rural area, Himachal Pradesh	858 (10 to 19 years, includes boys and girls) 30 clusters from two blocks	None	Not provided	Serum iron levels, vitamin B12
Rajaratnam et al 2000 [37] Survey	Rural area, Tamil Nadu	288 (13 to 19 years, girls) 20 clusters from two blocks	None	44.8%	Prevalence of anaemia
Sundaresan et al 2011 [39] Survey	Semi-urban area, Tamil Nadu	900 (8 to 16 years, includes both boys and girls) 10 schools in semi-urban area	None	Total 55.2% in boys and girls	Prevalence of anaemia and its correlation with BMI
Sukapriya et al 2015 [54] Survey	Urban area, Tamil Nadu	100 (17 to 18 years, girls) All participants were from one college.	None	44%	Prevalence of anaemia
Verma et al 2004 [55]	Urban area, Gujarat	1153 (6 to 18 years, girls)	None	81.8%	Prevalence of anaemia

Survey		15 slums in one city Ahmedabad			
Kotecha et al 2009 [56] Survey	Rural, urban and tribal areas, Gujarat	2797 girls in baseline study (12 to 19 years, girls). 2762 girls in supplementation assessment study. 30 communities in urban, trial and rural areas of Gujarat	None	74.7%	Prevalence of anaemia, iron supplementation, post-intervention assessment
Sinha et al 2014 [43] Survey	Rural area, Uttar Pradesh	254 (10 to 13 years, girls) 1 school in rural region	None	57.4%	Prevalence and chi square test
Singh et al 2006 [44] Survey	Urban area, Uttar Pradesh	400 (10 to 19 years, girls) Two urban slums in Lucknow city	None	56%	Prevalence of anaemia and signs of vitamins deficiencies
Agrawal et al 2003 [57] Survey	Urban area, Delhi	2088 (10 to 17 years, girls) Four schools in Delhi city	None	49.3%	Prevalence of anaemia and iron supplementation assessment
Biradar et al 2012 [58] Survey	Rural area, Karnataka	840 (10 to 19 year, girls) Villages under one primary health centre, no details provided.	None	41.8%	Anaemia prevalence
Rati et al 2014 [59] Survey	Rural area, Karnataka	300 (13 to 16 years, girls) Schools in rural region	None	80%	Anaemia prevalence
Gupta et al 2015 [46]	Rural and urban areas,	86 (7 years and above,	None	86%	Prevalence of

Survey	West Bengal	includes both boys and girls) Four villages and one municipal ward			anaemia and BMI
Bulliyy et al 2007 [47] Survey	Urban and rural areas, Orissa	1937 (11 to 19 years, girls) Three districts from Orissa	Age, BMI, mid upper arm circumference, education, family income	96.5%	Prevalence of anaemia, linear regression to study risk factors.
Peter et al 2012 [60] Survey	Urban and rural areas, Haryana	735 (16 to 30 years, girls) In 1 college, Bhodia city, Haryana	None	98%	Prevalence of anaemia, BMI
Naidu et al 2015 [61] Survey	Rural and urban areas, Andra Pradesh	628 (10 to 15 years, girls) Government schools in Tirupati region	None	98%	Prevalence and Chi square test
Bharati et al 2009 [36] National survey	Rural and urban areas at national level covering 35 states	177, 670 girls (from 35 Indian states, national survey)	Age, education, area of residence and caste/religion	National prevalence 89%	Prevalence of anaemia and regression analyses to study risk factors of anaemia

Table 2: Published studies on anaemia involving adolescent girl population from Maharashtra state of India (14 studies)

Author, publication year and study type	Study location in Maharashtra (city/district)	Sample size and age group	Risk factors of studied	Anaemia prevalence in adolescent girls unless indicated differently	Additional information/analysis
Majumdar et al 2012 [49] Survey	Urban area, Pune city	243 (16 to 20 years, girls) One college in Pune city	None	45.7%	Prevalence of anaemia and personal hygiene evaluation
Deshpande et al 2013 [62] Survey	Urban area, Pimpari, Pune district	1000 (12 to 15 years, girls) Schools in Pimpari city	None	71.1%	Prevalence of anaemia, chi square test
Patil et al 2009 [63] Survey	Rural area of Ratnagiri district	620 (10 to 19 years, girls) Random sampling from 168 villages	None	41.9%	Prevalence of anaemia and related health complaints
Bhanushali et al 2011 [64] Survey	Urban area, Mumbai city	387 (10 to 16 years, girls) One school in Mumbai city	None	26.8%	Prevalence of anaemia
Patil et al 2014 [65] Trial	Urban area of Karad city, Satara district	103 (11 to 18 years, girls) Slum area of Karad city	None	88%	Prevalence of anaemia and iron supplementation assessment
Deshmukh et al 2008 [66]	Urban, rural, tribal region of Nasik district	360 (14 to 18 years, girls)	None	65.3%	Prevalence of anaemia and iron

Trial		Random sampling in the district 30 clusters			supplementation assessment
Jawarkar et al 2015 [48] Trial	Urban area of Amravati city	350 (13 to 18 years, girls) One school in Amravati city	None	55%	Prevalence of anaemia and iron supplementation assessment
Chaudhary et al 2008 [50] Survey	Urban area of Nagpur city	296 (10 to 19 years, girls) Urban slum in Nagpur city	None	35.1%	Prevalence of anaemia
Dongre et al 2011 [67] Survey	Rural area of Wardha district	260 (12 to 19 years, girls) 23 villages in Wardha district	None	72.8%	Prevalence of anaemia and iron supplementation assessment
Joshi et al 2013 [68] Trial	Urban area of Nagpur city	120 (10 to 19 years, girls) Samples were selected from out patient department of medical college hospital	None	Before intervention 100% girls were anaemic After intervention 75% were anaemic	Prevalence of anaemia and iron supplementation assessment
Kulkarni et al 2012 [51] Survey	Urban area of Nagpur city	272 (10 to 19 years, girls) Samples were selected from out patient department of medical college hospital	None	90.1%	Prevalence of anaemia
Dongre et al 2011 [69] Survey	Rural area of Wardha district	Baseline 1270 (10 to 19 years, includes both boys and girls)	None	Total 83% in boys and girls	Prevalence of anaemia, iron supplementation assessment, personal

		Participants from 20 residential schools near Wardha city			hygiene, infections, oral health
Patle et al 2015 [70] Survey	Rural area of Nagpur district	600 (10 to 19 years, girls) Rural service area of a medical college, Nagpur	None	91%	Prevalence of anaemia and chi square test
Kuril et al 2015 [52] Trial	Hospital, Paithan city, Aurangabad District	378 (10 to 19 years, girls) Outpatient department of a medical college in Paithan	None	68.5%	Prevalence of anaemia and chi square test

3.2.2 Survey purpose

Though adolescent anaemia is a common health condition in India, limited data is available on its risk factors, thus existing prevention or treatment strategies are underdeveloped [46]. Individual factors such as age, dietary intake, and family resources may have a role in developing anaemia at young age. Village level risk factors such as healthcare access, water and sanitation facilities, transport services are important to consider particularly in rural Indian setting. However, no village level risk factors are examined in the published literature.

Therefore, we conducted a cross-sectional study involving 1,010 adolescent girls; the first conducted in Marathwada region, and the largest from rural Maharashtra, India. This detailed inquiry aimed to address current evident gaps in causes of anaemia in Indian adolescent girls, hence this should be helpful in planning future clinical investigations and public health interventions.

3.3 Methods

3.3.1 Study objectives

A cross sectional survey was conducted with a primary objective to investigate the prevalence and risk factors of anaemia in 13 to 17 year old adolescent girls from 35 villages of Osmanabad district, Maharashtra, India (approximately 65,000 population). Participants were recruited using the eligible lists prepared based on fulfilling the following two main conditions. Eligible list preparation process is outlined earlier in the chapter 2 (page number 38 to 42).

3.3.2 Eligibility criteria

1. Current age was between 13 to 17 years
2. Resident of the study villages (Appendix 1 study village list)

Participation was open to both, girls going to school and those who had dropped out. Study villages were organised in an ascending order based on their population to recruit the first 1000 participants. As described in detail in the Chapter 2 (page number 38 to 42), the research is planned as a feasibility study with a specific age range thus no formal sample size calculation was performed.

The secondary objective of the study was to compare haemoglobin measurements obtained from the Sahli's hemometer technique with the non-invasive haemoglobin technology (NBM 200). Eligibility criteria were the same as above. Thus, Hb was measured by the two methods, (a) Sahli's hemometer technique, and (b) NBM 200 device.

3.3.3 Data collection

The primary investigator (PI) and an assistant worked with the network of village health workers (VHWs) across the field area to contact eligible participants. Field based data collection procedure is described earlier in detail (page number 38 to 42). Two levels of data were collected: individual participant data and village profile data (Appendix 6 data collection forms).

Individual participant data

A trained project assistant and the PI administered individual data collection forms and obtained anthropometric measurements with the help of a nurse appointed for the MAS project.

A piloted data collection form was used to obtain sociodemographic information, medical history, iron and other supplements intake, 7-day dietary food frequency recall, family assets and menstrual history data as self-reported by participants, in accordance with the Standard Operating Procedures (Appendix 8). Systemic diseases such as malaria or other conditions that could influence Hb levels were assessed based on medical history (recent 12 months medication/hospitalisation), and clinical examination conducted at the time of data collection by the PI.

Height and Mid Upper Arm Circumference (MUAC) of the dominant hand were recorded using measuring tapes, and weight was recorded using a digital weighing machine (OMRON healthcare). MUAC was recorded using the circumference measured at the mid point between tip of elbow and the tip of shoulder. MUAC of < 22 cm was used as a recommended cut-off for the identification of malnutrition [71].

Village profile data

Village profile had information on village based healthcare facilities, infrastructure such as water supplies and transport resources. It was collected from HMF's village health workers (VHWs), government nurse and local government office, as explained previously (page number 46).

3.3.4 Haemoglobin investigations and anaemia assessment

Capillary haemoglobin (Hb) investigation was performed on the third finger of the non-dominant hand by a qualified laboratory technician under aseptic protocol using the Sahli's hemometer technique. A sterile, disposable needle was used in a sitting position. The hemometer uses Sahli's pipette with N/10 hydrochloric acid and blood sample, resulting in formation of acid hematin. Distilled water is then added to match the colour of hematin with adjacent colour comparator, and the exact match gives Hb reading as indicated on the pipette. Additional details of this procedure are outlined in the appendix 8. This is a well-establishment technique and also recommended by the Government of India to conduct Hb assessments particularly in rural setting [72].

Data collection and Hb investigations were conducted in the dry season (January to May), when temperature fluctuations were minimal reducing any environmental influence on Hb measurements [73]. For each participant, technician and the PI agreed on Hb levels showed on the colour comparator of the hemometer to reduce any subjectivity. The technician had an education background in laboratory services with four years of experience, and also received necessary research training by the PI and HMF hospital consultants.

Anaemia was defined as an Hb level below 12.0 g/dl [74]. Further classification into mild, moderate and severe anaemia was made based on haemoglobin level to facilitate post-investigation advice and referral [74]:

a. Severe anaemia when Hb is 7.9 g/dl and below
b. Moderate anaemia when Hb is between 8.0 g/dl and 10.9 g/dl
c. Mild anaemia when Hb is between 11.0 g/dl and 11.9 g/dl

d. Non-anaemic when Hb is 12.0 g/dl and above

All participants were defined using outlined categories based on the Hb estimates provided by Sahli's hemometer. For each participant only one Sahli's measurement was obtained. The same was reported on the health card provided to participants after complete data collection. While the Hb measurements obtained from the non-invasive device (NBM 200) were recorded for research purposes, and were not provided to participants. Data collection and both tests (Hemometer and NBM200) were performed in the same visit. Information about the NBM 200 technology is outlined below.

3.3.5 Non-invasive Hb measurement technology

Overview

The NBM 200 (Orsense Ltd, Nes-Ziona, Israel) is a portable non-invasive device (operates on electricity or batteries) that provides Hb measurements in 60 to 100 seconds [10]. NBM 200 has the advantage of not requiring material supplies (such as chemicals or needles) or post-testing bio-waste management services. The device provides the benefit of non-invasive testing that is not reliant on a skilled technician in areas where diagnostic facilities are limited. NBM 200 performs painless testing and also improves patient comfort.

The device has two parts (a) battery operated main device, and (b) finger probe (Figure 8). The probe gives an infrared red coloured light, which passes through the skin, and at the same time the probe applies pressure occluding blood vessels to measure Hb in the RBCs [10].

Figure 8: NBM 200 Device (main unit and a finger probe)



Figure source: Orsense Ltd www.orsense.com [10]

NBM 200 applications and study purpose

The existing evidence-base involves comparisons of the non-invasive technology with venous blood analysers or automated finger prick devices such as HemoCue (ANGELHOLM, Sweden) [75]. However, these methods are not commonly used in rural India mainly because of high operational costs and limited healthcare infrastructure. HemoCue requires a finger prick sample, and is costly compared to Sahli's method due to the initial investment for the device (300 to 600 GBP), and higher recurring costs for testing materials compared to Sahli's technique [30]. Operational costs for each HemoCue test is about 50 Indian Rupees (0.5 GBP), which is twice the cost of the Sahli's method [30]. Similarly, automated haematology analysers are expensive (generally 4000 GBP onwards), and require venous blood withdrawal as well as advanced laboratory infrastructure and trained personnel. A single automated analyser test costs between 100 to 350 Indian Rupees (1.0 to 3.5 GBP) [9,30]. While the initial cost of a non-invasive device such as NBM 200 is also high (about 1100

GBP), when used on a larger scale (5000 tests and above), each test costs about 20 to 25 Indian rupees, which is similar with the cost of Sahli's hemometer. Moreover, the NBM 200 could be used in the community setting on a larger scale by a healthcare auxiliary. Therefore, we assessed NBM 200 by comparing with Sahli's hemometer to investigate the application of the non-invasive technique in rural areas of Maharashtra state of India. The NBM 200 device was sponsored by the Clinical Translational Research Priority Group Award for International Collaboration, University of Nottingham, UK (Grant code: A2RN72).

3.3.6 Statistical analysis

All data were recorded manually on printed forms in the field area, and then the same were entered in computer to develop electronic records. Data were cleaned and checked wherever required.

Analyses were performed using Stata 13.1 (StataCorp, College Station, Texas, USA). Initially frequencies and proportions were calculated to understand the dataset. Linearity of variables such as age was calculated wherever necessary before considering the variable as a continuous measure. In all analyses statistical significance was defined as p value less than 0.05. Overview of statistical analysis is presented in figure 10.

Analysis was grouped in the three sections:

1. Describing the prevalence and severity of anaemia in adolescent population

2. Risk factor associated with anaemia- both individual and village level risk factors were analysed.

3. Comparison of non-invasive haemoglobin technology (NBM 200) by comparing with the Sahli's hemometer measurements.

1. Anaemia prevalence and severity

Those with Hb less than 12.0 g/dl were identified as anaemic, and the prevalence was calculated in the studied population. Further categorisation in mild, moderate and severe was performed as outlined earlier to refer participants to appropriate health facility.

2. Risk factors associated with anaemia

Individual risk factors

Regression techniques were used to evaluate risks associated with anaemia [76].

Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the association of each risk factor with anaemia status (binary categorical variable such as anaemic/not anaemic). Linear regression was used to assess the association between each risk factor with haemoglobin level, and results were presented in regression coefficients with 95% CI. During both regression techniques unadjusted analysis was performed as an initial step where all variables were analysed. Variable such as 7-days dietary recall was condensed into 2 categories in order to analyse the data for regression purposes. For example, dietary data were collected based on a weekly consumption pattern using the following four categories: Never, 1-2 times a week, 3-6 times a week and Everyday. The four categories were condensed in two (≤2 times a week, and ≥3 times week). The

process provided sufficient participants in each category to conduct regression analysis.

After the initial unadjusted analyses, a multivariable data analysis approach was developed. In the adjusted analysis individual risk factors based on their theoretical relationship with anaemia, and also with an understanding of the field setting, were assigned to one of three groups: individual health factors, dietary factors and socioeconomic factors (Figure 9), making set of variable more restricted. Three multivariate models including mutual adjustment of within-group factors only were constructed. A fourth model was then allowed for full adjustment for all risk factors from this multivariable model. During this model development, we also assessed multicollinearity of all factors using a covariate correlation matrix, and the variance inflation factor (VIF) [77]. Multicollinearity was only found between height, weight, and calculated body mass index; therefore, MUAC was the only anthropometric measurement included in the model (VIF <4). Additional information on each variable selection is outlined in detail in appendix 9.

Figure 9: Risk factor classification for multivariable modelling

Individual health variables (group 1): Age, Mid upper-arm circumference (MUAC), Currently consuming iron folic acid supplements, Attained menarche.

Dietary variables (group 2): 7-day diet recall of daily intake (milk, green leafy vegetables, sprouts, pulses-lentils, fruits/fruit juices, eggs, chicken and goat meat)

Socioeconomic variables (group 3): Current education status, Siblings (elder/younger), Religion/caste and Farming land.

Village level risk factors

Unadjusted analyses (logistic and linear regression) were conducted to evaluate village level risk factors associated with anaemia, and then a fully adjusted analysis was performed. Risk factors were limited to nine covariates, thus a fully adjusted model was preferred. The covariates were selected based on the understanding of the field area, and also their relevance to adolescent anaemia (Appendix 10).

3. Comparison of non-invasive haemoglobin technology (NBM 200) with Sahli's hemometer technique

The Sahli's hemometer measurements were considered as the reference in the survey, and those were compared with haemoglobin estimates generated from the NBM 200. Initially Spearman's coefficient correlation was calculated and then the Bland-Altman analysis was performed to measure level of agreement between two techniques [78].

Additional analyses included sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculations to compare NBM 200 with the reference method.

- Sensitivity: The proportion of true positives correctly identified by NBM 200.
- Specificity: The proportion of true negatives correctly predicted by NBM 200.
- Positive predictive value (PPV): The proportion of NBM 200 anaemia positive participants for whom the anaemia diagnosis was confirmed by Sahli's hemometer.
- Negative predictive value (NPV): The proportion of NBM 200 anaemia negative participants who were non-anaemic as per the Sahli's hemometer.

Area Under Receiving Operating Characteristics (AUROC) was analysed to examine performance of the NBM 200. To allow for possible confounding by environmental temperatures, sensitivity analysis was conducted using data from two consecutive months to assess variations in Hb levels using Bland-Altman analysis.

Figure 10: Overview of statistical analysis

Individual risk factors

- Unadjusted analysis for all individual factors collected during the data collection.
- Multivariable model approach was developed where selected factors were included in the model (within group and fully adjusted models were presented).
- Logistic and linear regressions were conducted.

Village level risk factors

- Unadjusted analysis of village factors relevant to adolescent health in the study area.
- Fully adjusted model where all factors listed in the unadjusted analysis were included.
- Logistic and linear regressions were conducted.

Sahli's hemometer and NBM 200 comparison

- Hb measurements from both techniques were compared using correlation, Bland-Altman analysis, sensitivity, specificity, NPV, PPV, and AUROC tests.
- Sahli’s hemometer was considered as the reference method.

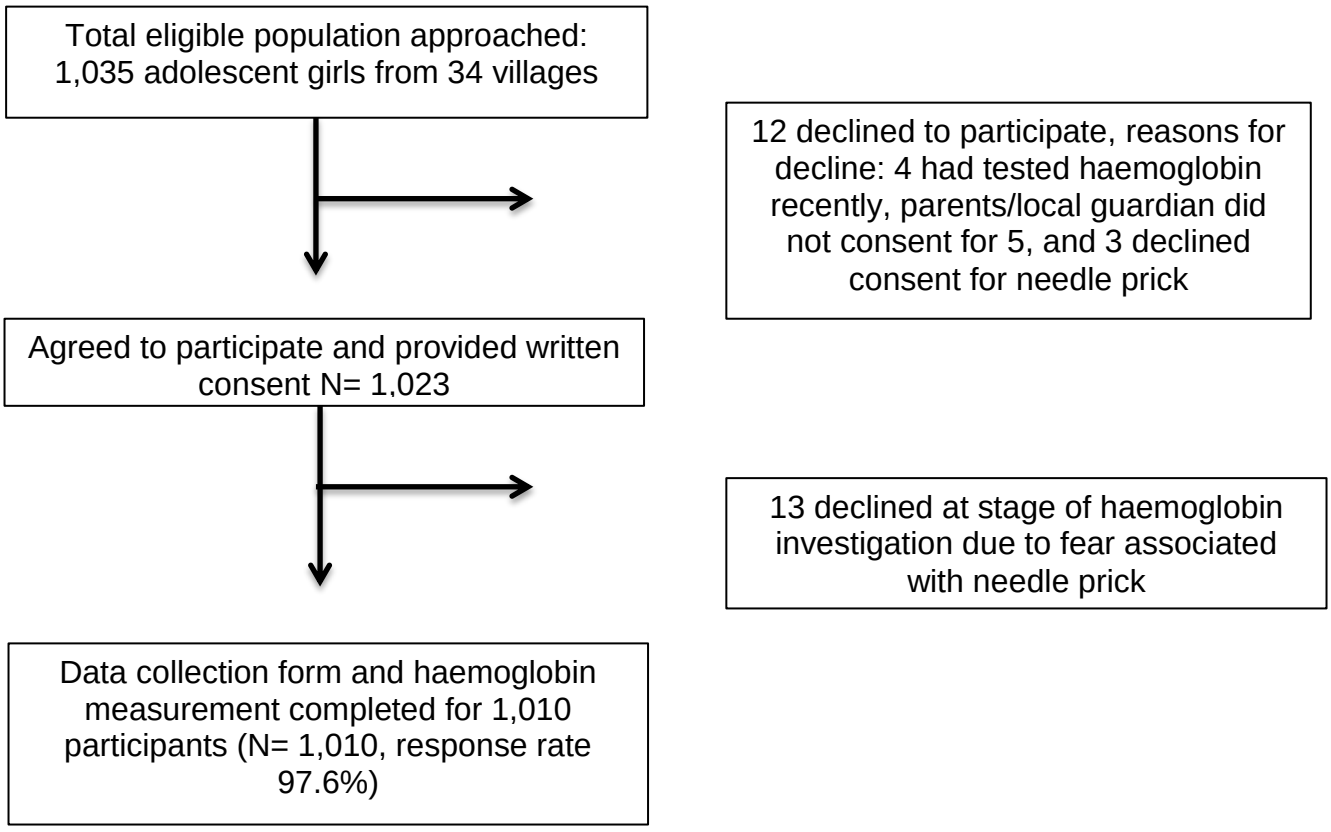
3.4 Results

The results are presented in the four sections: summary, individual risk factors associated with anaemia, village level risk factors associated with anaemia, and Non-invasive haemoglobin technology assessment.

3.4.1 Prevalence and severity of anaemia

We approached 1,035 adolescent girls from the first 34 villages (Appendix 1 study village list). Of which only 25 (2%) did not participate in full data collection (Figure 11). In the risk factors analysis both logistic and linear regressions were conducted involving data from 1,010 study participants. While, the non-invasive device (NBM 200) comparison involved 766 participants.

Figure 11: Flow chart of recruitment process and final study population



Analysis from 1,010 samples showed a very high prevalence of anaemia (87% were anaemia, 95% Confidence Intervals [CI]: 85.0 to 89.2). Severe anaemia was observed in 5% (N=48), moderate anaemia in 65% (N=659), and mild anaemia in 17% (N=173). No participants reported systemic diseases such as malaria or other conditions that could have influenced Hb levels. None were admitted in hospital or received blood transfusions in the 12 months prior to the study. Hb levels were normally distributed for the 1,010 participants (Figure 12), and ranged from 5.0 g/dl to 14.0 g/dl with a mean of 10.1 g/dl (standard deviation (SD): 1.3, 95% CI: 10.0 to 10.2).

Figure 12: Histogram of haemoglobin distribution in adolescent girls (N=1010)

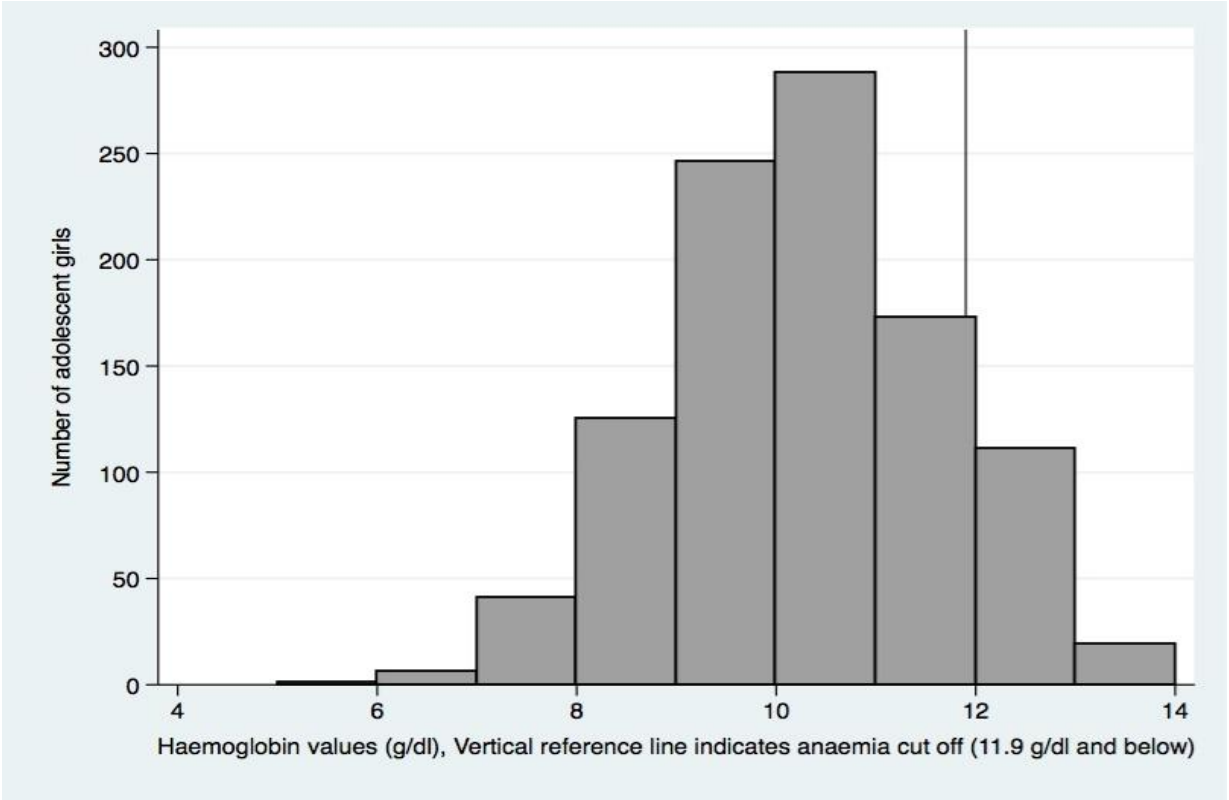


Table 3 shows anaemia prevalence according to studied risk factors. Anaemia prevalence did not vary substantially by age. Majority of participants were underweight (67% with BMI < 18.5 kg/m^2). Anaemia was more prevalent among girls with MUAC < 22 cm. Less than 10% were on iron folic acid supplements. 42% of participants were vegetarians, 18% eggetarians and 40% non-vegetarians, however, less than 10% consumed meat 3 or more times a week. We observed an overall low consumption (≤2 times per week) of milk, eggs, green leafy vegetables, bean sprouts and fruits/fruit juices, whereas pulses/lentils and rice were more frequently included in the diet (Table 3).

3.4.2 Individual risk factors associated with adolescent anaemia

About 9% were school dropouts, most had older or younger siblings, the majority (61%) were from the Hindu open castes, and 78% had parents who owned farming land with 36% having more than five acres of land. Of these factors, current education, religion category and farm land possession were associated with anaemia in the unadjusted analyses.

The only dietary risk factors showing statistically significant associations with anaemia in unadjusted analysis were higher consumption of milk, green leafy vegetables, fruits/fruit juices and goat meat.

The unadjusted analysis is reported in table 3.

Table 3: Characteristics of study population with unadjusted analyses (n= 1010 participants, unless noted differently)

Characteristics	Participants (%)	Non-anaemic participants (%)	Anaemic participants (%)	Coefficient with 95% confidence interval	Linear regression p value	Odds ratio with 95% confidence interval	Logistic regression p value
Age in years							
13	207 (20.5)	25 (19.2)	182 (20.6)	-0.00 (-0.06 to 0.04)	0.74	1.09 (0.96 to 1.25)	0.15
14	229 (22.6)	39 (30.0)	190 (21.5)				
15	199 (19.7)	26 (20.0)	173 (19.6)				
16	173 (17.1)	21 (16.1)	152 (17.2)				
17	202 (20)	19 (14.6)	183 (20.8)				
Current education status							
School going	921 (91.1)	110 (84.6)	811 (92.1)	Ref	-	Ref	-
School dropout	89 (8.8)	20 (15.3)	69 (7.8)	0.24 (-0.04 to 0.53)	0.09	0.46 (0.27 to 0.79)	0.005
Temporary paid work (within farm/shops/factories)							
No	930 (92)	112 (86.1)	818 (92.9)	Ref	-	Ref	-
Yes	80 (8)	18 (13.8)	62 (7.0)	0.16 (-0.14 to 0.47)	0.29	0.47 (0.26 to 0.82)	0.009
Handedness							
Right handed	971 (96.1)	126 (96.9)	845 (96.0)	Ref	-	Ref	-
Left handed	39 (3.8)	4 (3.0)	35 (3.9)	-0.02 (-0.45 to 0.40)	0.92	1.30 (0.45 to 3.73	0.62
At least one elder sibling (immediate brother/sister)							
No	276 (27.3)	37 (28.4)	239 (27.1)	Ref	-	Ref	-
Yes	734 (72.6)	93 (71.5)	641 (72.8)	-0.12 (-0.30 to 0.06)	0.19	1.06 (0.70 to 1.60)	0.75
At least one younger sibling (immediate brother/sister)							
No	337 (33.3)	42 (32.3)	295 (33.5)	Ref	-	Ref	-
Yes	673 (66.6)	88 (67.6)	585 (66.4)	0.12 (-0.04 to 0.30)	0.15	0.94 (0.63 to 1.40)	0.78

Mid-upper arm circumference of dominant hand (MUAC)							
> 22 cm and above	503 (49.8)	79 (60.7)	424 (48.1)	Ref	-	Ref	-
< 22 cm	507 (50.2)	51 (39.2)	456 (51.8)	-0.36 (-0.52 to -0.19)	<0.001	1.66 (1.14 to 2.42)	0.008
Body Mass Index (BMI) in kg/m^2							
Normal (BMI 18.5 to 24.9)	330 (32.6)	42 (32.1)	288 (32.7)	Ref	-	Ref	-
Underweight (BMI < 18.5)	671 (66.4)	87 (66.9)	584 (66.3)	-0.29 (-0.47 to -0.12)	0.001	0.97 (0.65 to 1.45)	0.91
Overweight (BMI 25.0 to 29.9)	7 (0.6)	1 (0.7)	6 (0.6)	0.02 (-0.98 to 1.02)	0.96	0.87 (0.10 to 7.44)	0.90
Obese (30.0 and above)	2 (0.2)	0	2 (0.2)	0.19 (-1.67 to 2.05)	0.84	Omitted	-
Any diagnosed disease							
No	1005 (99.5)	130 (100)	875 (99.4)	Ref	-	Omitted	-
Yes *	5 (0.5)	0	5 (0.5)	0.14 (-1.03 to 1.33)	0.80	-	-
Consumed Iron Folic Acid supplement in the past							
No	520 (51.4)	70 (53.8)	450 (51.1)	Ref	-	Ref	-
Yes	469 (46.4)	57 (43.8)	412 (46.8)	-0.33 (-0.50 to -0.16)	<0.001	1.12 (0.77 to 1.63)	0.53
Do not remember	21 (2.08)	3 (2.3)	18 (2.05)	-0.05 (-0.64 to 0.52)	0.84	0.93 (0.26 to 3.25)	0.91
Currently taking Iron Folic Acid supplements							
No	917 (90.7)	128 (98.4)	789 (89.6)	Ref	-	Ref	-
Yes **	93 (9.2)	2 (1.5)	91 (10.3)	-0.55 (-0.91 to 0.11)	0.12	3.92 (0.52 to 29.19)	0.18
Currently taking calcium supplements							
No	1008 (99.8)	130 (100)	878 (99.7)	Omitted	-	Omitted	-
Yes ***	2 (0.2)	0	2 (0.2)	-	-	-	-
Currently taking any other medication including multi-vitamins							
No	983 (97.3)	129 (99.2)	854 (97.0)	Ref		Ref	-

Yes ^	27 (2.6)	1 (0.7)	26 (2.9)	-0.39 (-0.91 to 0.11)	0.12	3.92 (0.52 to 29.19)	0.18
Religion and category (open/reserve)							
Hindu open category	623 (61.6)	66 (50.7)	557 (63.3)	Ref	-	Ref	-
Hindu reserve category	321 (31.78)	60 (46.1)	261 (29.6)	-0.08 (-0.12 to -0.03)	<0.001	0.51 (0.35 to 0.75)	0.001
Muslim	66 (6.5)	4 (3.0)	62 (7.0)	0.04 (-0.03 to 0.12)	0.29	1.83 (0.64 to 5.21)	0.25
House structure							
Permanent (pucca- concrete slab rooftop)	4 (0.4)	2 (1.5)	2 (0.2)	Ref	-	Ref	-
Semi-Permanent (semi pucca- metal sheet rooftop)	882 (87.3)	106 (81.5)	776 (88.1)	-0.17 (-1.49 to 1.13)	0.78	7.32 (1.02 to 52.51)	0.04
Temporary (kutcha- wood/straw rooftop)	124 (12.2)	22 (16.9)	102 (11.5)	0.35 (-0.98 to 1.68)	0.60	4.6 (0.61 to 34.72)	0.13
Parents possess farming land							
More than 5 acres	368 (36.4)	35 (26.9)	333 (37.8)	Ref	-	Ref	-
Less than 5 acres	420 (41.5)	56 (43.0)	364 (41.3)	-0.06 (-0.28 to 0.15)	0.57	0.68 (0.30 to 0.80)	0.005
No land	222 (21.9)	39 (30.0)	183 (20.8)	0.00 (-0.21 to 0.23)	0.06	0.49 (0.43 to 1.06)	0.09
Parents possess livestock (cows/buffaloes/bulls/sheep/goats/chickens)							
No	284 (28.1)	48 (36.9)	236 (26.8)	Ref	-	Ref	-
Yes	726 (71.8)	82 (63.0)	644 (73.1)	-0.03 (-0.22 to 0.14)	0.68	1.59 (1.08 to 2.35)	0.01
Parents possess television set							
No	415 (41.0)	58 (44.6)	357 (40.5)	Ref	-	Ref	-
Yes	595 (58.9)	72 (55.3)	523 (59.4)	-0.04 (-0.21 to 0.12)	0.59	1.18 (0.81 to 1.71)	0.38
Parents possess mobile phone							
No	63 (6.2)	14 (10.7)	49 (5.5)	Ref	-	Ref	-
Yes	947 (93.7)	116 (89.2)	831 (94.4)	-0.17 (-0.51	0.32	2.04 (1.09 to	0.02

				to 0.17)		3.82)	
Registered under the below poverty line families (BPL)							
No	635 (62.8)	65 (50.0)	570 (64.7)	Ref	-	Ref	-
Yes	375 (37.1)	65 (50.0)	310 (35.2)	0.17 (-0.00 to 0.34)	0.05	0.54 (0.37 to 0.78)	0.001
Dietary preference							
Vegetarian	428 (42.3)	52 (40.0)	376 (42.7)	Ref	-	Ref	-
Eggetarian	184 (18.2)	24 (18.4)	160 (18.1)	-0.09 (-0.32 to 0.14)	0.43	0.92 (0.54 to 1.54)	0.75
Non vegetarian	398 (39.4)	54 (41.5)	344 (39.0)	0.00 (-0.17 to 0.19)	0.92	0.88 (0.58 to 1.32)	0.54
Diet recall (last seven days history)							
Pure milk (not in the form of tea/coffee)							
≤ 2 times a week	792 (78.4)	93 (71.5)	699 (79.4)	Ref	-	Ref	-
≥ 3 times a week	218 (21.5)	37 (28.4)	181 (20.5)	0.13 (-0.06 to 0.33)	0.19	0.65 (0.42 to 0.98)	0.04
Green leafy vegetables							
≤ 2 times a week	723 (71.5)	83 (63.8)	640 (72.7)	Ref	-	Ref	-
≥ 3 times a week	287 (28.4)	47 (36.1)	240 (27.2)	0.03 (-0.14 to 0.22)	0.68	0.66 (0.44 to 0.97)	0.03
Bean sprouts							
≤ 2 times a week	967 (95.7)	126 (96.9)	841 (95.5)	Ref	-	Ref	-
≥ 3 times a week	43 (4.2)	4 (3.0)	39 (4.4)	-0.14 (-0.55 to 0.26)	0.48	1.46 (0.51 to 4.15)	0.47
Pulses-lentils							
≤ 2 times a week	307 (30.4)	41 (31.5)	266 (30.2)	Ref	-	Ref	-
≥ 3 times a week	703 (69.6)	89 (68.4)	614 (69.7)	0.02 (-0.15 to 0.20)	0.79	1.06 (0.71 to 1.58)	0.76
Fruits/Fruit juices							
≤ 2 times a week	774 (76.6)	74 (56.9)	700 (79.5)	Ref	-	Ref	-
≥ 3 times a week	236 (23.3)	56 (43.0)	180 (20.4)	0.48 (0.28 to	<0.001	0.33 (0.23 to	<0.001

				0.67)		0.49)	
Rice							
≤ 2 times a week	103 (10.2)	7 (5.3)	96 (10.9)	Ref	-	Ref	-
≥ 3 times a week	907 (89.8)	123 (94.6)	784 (89.0)	0.11 (-0.16 to 0.38)	0.42	0.46 (0.21 to 1.02)	0.05
Eggs							
≤ 2 times a week	980 (97.0)	125 (96.1)	855(97.1)	Ref	-	Ref	-
≥ 3 times a week	30 (2.9)	5 (3.8)	25 (2.8)	-0.00 (-0.49 to 0.48)	0.98	0.73 (0.27 to 1.94)	0.53
Chicken							
≤ 2 times a week	1001 (99.1)	128 (98.4)	873 (99.2)	Ref	-	Ref	-
≥ 3 times a week	9 (0.89)	2 (1.5)	7 (0.8)	0.30 (-0.58 to 1.18)	0.50	0.51 (0.10 to 2.49)	0.40
Goat meat							
≤ 2 times a week	1005 (95.5)	127 (97.6)	878 (99.7)	Ref	-	Ref	-
≥ 3 times a week	5 (0.5)	3 (2.3)	2 (0.2)	1.27 (0.09 to 2.45)	0.03	0.09 (0.01 to 0.58)	0.01
Heard about anaemia and haemoglobin							
No	576 (57.0)	80 (61.5)	496 (56.3)	Ref	-	Ref	-
Yes	434 (42.9)	50 (38.4)	384 (43.6)	-0.18 (-0.34 to -0.01)	0.03	1.23 (0.84 to 1.80)	0.26
Tested haemoglobin before							
No	227 (22.4)	31 (23.8)	196 (22.2)	Ref	-	Ref	-
Yes	747 (73.9)	97 (74.6)	650 (73.8)	-0.17 (-0.37 to 0.02)	0.08	1.05 (0.68 to 1.63)	0.18
Do not remember	36 (3.5)	2 (1.5)	34 (3.8)	-0.49 (-0.96 to -0.01)	0.04	2.68 (0.61 to 11.75)	0.79
Aware about your recent haemoglobin levels							
No	477 (47.2)	81 (62.3)	396 (45.0)	Ref	-	Ref	-
Yes	399 (39.5)	37 (28.4)	362 (41.1)	-0.42 (-0.59 to -0.24)	<0.001	2.00 (1.32 to 3.02)	0.001

Do not remember	134 (13.2)	12 (9.2)	122 (13.8)	-0.31 (-0.57 to -0.05)	0.01	2.07 (1.09 to 3.94)	0.02
Attained menarche							
No	360 (35.6)	39 (30.0)	321 (36.4)	Ref	-	Ref	-
Yes	635 (62.8)	88 (67.6)	547 (62.1)	0.00 (-0.16 to 0.18)	0.93	0.75 (0.50 to 1.12)	0.17
Declined	15 (1.4)	3 (2.3)	12 (1.3)	0.41 (-0.28 to 1.10)	0.24	0.48 (0.13 to 1.79)	0.28
Know the age at menarche (n=629)							
No	592 (94.1)	86 (100)	506 (93.1)	Ref	-	Omitted	-
Yes	37 (5.8)	0	37 (6.8)	-0.34 (-0.81 to 0.11)	0.14	-	-
Missed school due to menstrual cycle (n=629)							
No	524 (83.3)	69 (80.2)	455 (83.7)	Ref	-	Ref	-
Yes	105 (16.6)	17 (19.7)	88 (16.2)	-0.11 (-0.40 to 0.17)	0.44	0.84 (0.46 to 1.55)	0.59

Table 3 footnotes:

- * Diagnosed diseases *YES:* 4 had Epilepsy and 1 had speech disorder.
- ** Currently taking IFA: IFA prescribed at school to 87 girls, government nurse/healthcare worker prescribed to 3 participants, and 3 girls obtained on consultation with a private doctor.
- *** Taking calcium *YES:* prescribed by private doctor to 2 girls.
- ^ On any other medication *YES:* 14 participants did not know the exact name of medicine, 9 participants were having Mikronutrient supplement (medicine brand name, provided by school), 4 participants were consuming multivitamin syrup.
- For linear regression, haemoglobin values (g/dl) as a continuous measure (was the main outcome) and for logistic regression anaemic or non-anaemic status (was the main outcome). Ref indicates reference category.
- Age and age at menarche used as a continuous variable (after estimation of likelihood ratio) in regression.

Adjusted regression results: Individual risk factors associated with anaemia

The fully adjusted logistic regression is outlined in the table 4. This included individual health, dietary and socioeconomic factors selected using the multivariable modelling strategy described earlier (Figure 9).

Factors associated with increased odds of anaemia were, increasing year of age (OR 1.41 per year, 95% CI: 1.17 to 1.70, linear increase, p<0.001), and IFA supplementation (OR 5.68, 95% CI: 1.35 to 23.79, p=0.01). Larger MUAC (OR 0.51, 95% CI: 0.31 to 0.82 for ≥ 22cm compared with < 22cm, p=0.006), consumption of fruit/fruit juice or rice ≥3 times per week compared with less often (OR 0.35, 95% CI: 0.23 to 0.54, p<0.001, and OR 0.39, 95% CI: 0.17 to 0.91, p<0.001), and having dropped out of school (OR 0.47, 95% CI: 0.24 to 0.91, p=0.02) were all associated with reduced odds of anaemia. The effect estimates of the studied risk factors were roughly similar across all three models (unadjusted analysis, with group adjustment and a fully adjusted model).

The fully adjusted linear regression model (Table 5) showed that only larger MUAC (p<0.001), and higher fruits/fruit juice consumption (p<0.001) were significantly associated with increased Hb levels, whereas current IFA supplementation was associated with decreased Hb levels (p=0.002), however the sizes of these associations were small.

Table 4: Multivariable modelling, logistic regression results: individual risk factors associated with anaemia in adolescent girls (N= 1,010)

Characteristics	Unadjusted OR (95% CI)	Models adjusted for within-group factors only[1] OR (95% CI)	Fully adjusted model OR (95% CI)
Individual health variables			
Age in years	1.09 (0.96 to 1.25)	1.34 (1.13 to 1.58) **	1.41 (1.17 to 1.70) **
Mid-upper arm circumference of dominant hand (MUAC)			
< 22 cm	Ref	Ref	Ref
≥ 22 and above	0.60 (0.41 to 0.87) *	0.52 (0.33 to 0.81) **	0.51 (0.31 to 0.82) **
Currently consuming Iron Folic Acid supplements			
No	Ref	Ref	Ref
Yes	7.38 (1.79 to 30.33) *	7.52 (1.82 to 31.05) *	5.68 (1.35 to 23.79) *
Attained menarche			
No	Ref	Ref	Ref
Yes	0.75 (0.50 to 1.12)	0.65 (0.39 to 1.10)	0.78 (0.45 to 1.34)
Declined	0.48 (0.13 to 1.79)	0.45 (0.11 to 1.74)	0.43 (0.10 to 1.83)
Dietary variables (Ref ≤ 2 times a week)			
Pure milk (not in the form of tea/coffee)			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.65 (0.42 to 0.98) *	0.71 (0.46 to 1.10)	0.65 (0.41 to 1.02)
Green leafy vegetables			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.66 (0.44 to 0.97) *	0.73 (0.48 to 1.11)	0.69 (0.44 to 1.06)
Bean sprouts			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	1.46 (0.51 to 4.15)	2.42 (0.80 to 7.28)	2.59 (0.82 to 8.18)
Pulses-lentils			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	1.06 (0.71 to 1.58)	1.26 (0.83 to 1.92)	1.22 (0.79 to 1.88)
Fruits/Fruit juices			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.33 (0.23 to	0.35 (0.23 to	0.35 (0.23 to 0.54) **

	0.49) **	0.52) **	
Rice			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.46 (0.21 to 1.02)	0.45 (0.20 to 1.02)	0.39 (0.17 to 0.91) *
Eggs			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.73 (0.27 to 1.94)	0.96 (0.31 to 2.94)	0.93 (0.30 to 2.89)
Chicken			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.51 (0.10 to 2.49)	0.75 (0.09 to 6.26)	1.02 (0.13 to 7.82)
Goat meat			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.09 (0.01 to 0.58) *	0.11 (0.01 to 0.88)*	0.23 (0.02 to 2.15)
Socioeconomic variables			
Current education status			
School going	Ref	Ref	Ref
School dropout	0.46 (0.27 to 0.79) *	0.56 (0.32 to 0.99) *	0.47 (0.24 to 0.91) *
At least one elder sibling (immediate brother/sister)			
No	Ref	Ref	Ref
Yes	1.06 (0.70 to 1.60)	1.19 (0.76 to 1.86)	0.96 (0.60 to 1.56)
At least one younger sibling (immediate brother/sister)			
No	Ref	Ref	Ref
Yes	0.94 (0.63 to 1.40)	1.03 (0.67 to 1.58)	0.90 (0.57 to 1.43)
Religion and category			
Hindu open category	Ref	Ref	Ref
Hindu reserved category	0.51 (0.35 to 0.75) *	0.61 (0.40 to 0.94) *	0.75 (0.48 to 1.17)
Muslim	1.83 (0.64 to 5.21)	2.12 (0.73 to 6.11)	2.34 (0.78 to 6.99)
Parents possess farming land			
No land	Ref	Ref	Ref
Less than 5 acres	2.02 (1.24 to 3.31) *	1.56 (0.90 to 2.70)	1.74 (0.98 to 3.08)
More than 5 acres	1.38 (0.88 to 2.16)	1.10 (0.67 to 1.79)	1.12 (0.67 to 1.86)

Table 4 footnotes:

- * Indicates p<0.05 ** Indicates p<0.001
- [1] Models with within-group adjustments include only risk factors within that group, i.e. there are 3 models: the first with mutual adjustment only for

individual health factors, the second with mutual adjustment only for dietary factors and the third with mutual adjustment only for socioeconomic factors, as described in the figure 9.

- The fully adjusted model includes all risk factors in the table. Ref indicates reference category.

Table 5: Multivariable modelling, linear regression results: individual risk factors associated with haemoglobin level (g/dl) in adolescent girls (N= 1,010)

Characteristics	Unadjusted β coefficient (95% CI) Hb in g/dl	Models adjusted for within-group factors[1] only, β (95% CI)	Fully adjusted model β (95% CI) Hb in g/dl
Individual health variables			
Age in years	0.00 (-0.06 to 0.04)	-0.06 (-0.13 to -0.00)	-0.07 (-0.14 to 0.00)
Mid-upper arm circumference of dominant hand (MUAC)			
< 22 cm	Ref	Ref	Ref
≥ 22 and above	0.36 (0.19 to 0.52) **	0.49 (0.30 to 0 .68) **	0.46 (0.27 to 0.66) **
Currently consuming Iron Folic Acid supplements			
No	Ref	Ref	Ref
Yes	-0.55 (-0.83 to -0.26) **	-0.53 (-0.82 to -0.25) *	-0.44 (-0.72 to -0.15) **
Attained menarche			
No	Ref	Ref	Ref
Yes	0.00 (-0.16 to 0.18)	-0.14 (-0.37 to 0.08)	-0.17 (-0.40 to 0.5)
Declined	0.41 (-0.28 to 1.10)	0.26 (-0.42 to 0.95)	0.29 (-0.39 to 0.99)
Dietary variables (Ref ≤ 2 times a week)			
Pure milk (not in the form of tea/coffee)			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.13 (-0.06 to 0.33)	0.10 (-0.10 to 0.30)	0.08 (-0.11 to 0.28)
Green leafy vegetables	Ref	Ref	Ref
≤ 2 times a week			
≥ 3 times a week	0.03 (-0.14 to 0.22)	0.00 (-0.19 to 0.18)	0.00 (-0.17 to 0.19)
Bean sprouts			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	-0.14 (-0.55 to 0.26)	-0.27 (-0.69 to 0.13)	-0.25 (-0.66 to 0.15)
Pulses-lentils			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.02 (-0.15 to 0.20)	-0.03 (-0.21 to 0.15)	-0.01 (-0.19 to 0.16)
Fruits/Fruit juices			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.48 (0.28 to	0.47 (0.28 to	0.45 (0.25 to 0.64)

	0.67) **	0.67) **	**
Rice			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.11 (-0.16 to 0.38)	0.09 (-0.17 to 0.37)	0.14 (-0.13 to 0.41)
Eggs			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.00 (-0.49 to 0.48)	-0.11 (-0.63 to 0.40)	-0.09 (-0.61 to 0.42)
Chicken			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	0.30 (-0.58 to 1.18)	0.24 (-0.73 to 1.22)	0.21 (-0.75 to 1.18)
Goat meat			
≤ 2 times a week	Ref	Ref	Ref
≥ 3 times a week	1.27 (0.09 to 2.45) *	1.08 (-0.14 to 2.32)	0.76 (-0.45 to 1.99)
Socioeconomic variables			
Current education status	Ref	Ref	Ref
School going	Ref	Ref	Ref
School dropout	0.24 (-0.04 to 0.53)	0.22 (-0.07 to 0.52)	0.18 (-0.12 to 0.49)
At least one elder sibling (immediate brother/sister)			
No	Ref	Ref	Ref
Yes	-0.12 (-0.30 to 0.06)	-0.11 (-0.31 to 0.09)	-0.06 (-0.26 to 0.13)
At least one younger sibling (immediate brother/sister)			
No	Ref	Ref	Ref
Yes	0.12 (-0.04 to 0.30)	0.07 (-0.11 to 0.26)	0.10 (-0.08 to 0.29)
Religion and category			
Hindu open category	Ref	Ref	Ref
Hindu reserved category	0.18 (0.00 to 0.36) *	0.20 (0.00 to 0.40) *	0.13 (-0.06 to 0.33)
Muslim	-0.00 (-0.34 to 0.34)	0.02 (-0.32 to 0.37)	0.02 (-0.32 to 0.36)
Parents possess farming land			
No land	Ref	Ref	Ref
Less than 5 acres	0.00 (-0.21 to 0.23)	0.12 (-0.12 to 0.37)	0.09 (-0.14 to 0.34)
More than 5 acres	-0.06 (-0.28 to 0.15)	0.02 (-0.20 to 0.26)	0.06 (-0.16 to 0.29)

Table 5 footnotes:

- * Indicates p<0.05 ** Indicates p<0.001
- [1]Models with within-group adjustments include only risk factors within that group, i.e. there are 3 models: the first with mutual adjustment only for individual health factors, the second with mutual adjustment only for dietary

factors and the third with mutual adjustment only for socioeconomic factors, as described in figure 9.

- The fully adjusted model includes all risk factors in the table. Ref indicates reference category.

3.4.3 Village level risk factors associated with adolescent anaemia

Recruitment was done in the first 34 villages. The largest village 'Khudawadi' was not approached as the proposed sample size of 1000 was achieved. The risk factor analysis is based on 34 villages from the MAS field area.

Villages had similar social and health infrastructure. All villages had government nurses, however most of them were visiting only once a month (N= 29). Nine villages had government health centres, and only one had a primary health centre (PHC) with 24 hours emergency service. None had a centralised water purification facility, and all had limited transport services (mostly private operators). Ten villages had secondary schools (10th standard/Year 10, up to 15 years of age).

Mean haemoglobin was between 8 and 10 g/dl for the study participant population in most villages (N=18), with only one village having mean Hb over 12.0 g/dl (Figure 13). Rest villages (N=15) had mean Hb between 11 to 11.9 g/dl. No village was observed with mean below 8.0 g/dl.

Figure 13: Haemoglobin distribution in study villages (n=34 villages)

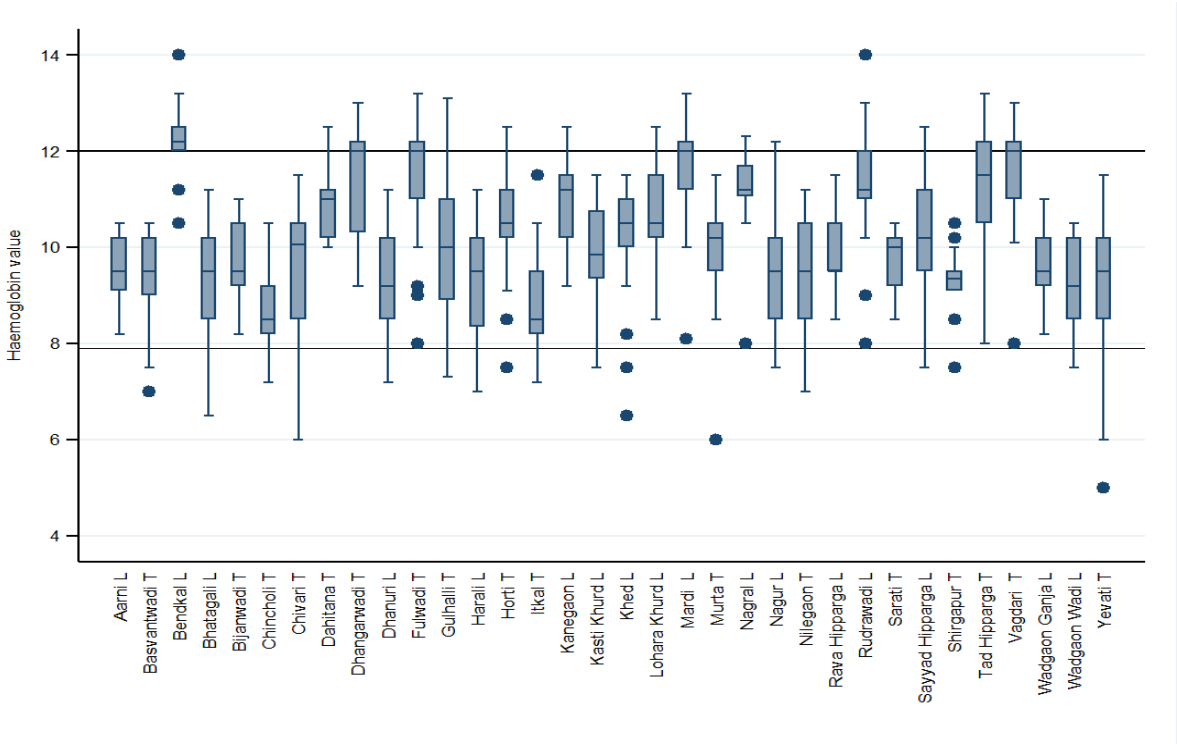


Figure 13 footnotes:

- Villages plotted on X axis and haemoglobin values on Y-axis. Village name is followed by block initials such as T for Tuljapur and L for Lohara block, as indicated on the X-axis.
- Y-axis horizontal reference lines: At 12.0 g/dl, area above indicates non-anaemic population, and the reference line at 7.9 g/dl and area below indicates severe anaemic population. Area between these two reference lines shows population with mild and moderate anaemia. Dots are outliers.

Village level risk factors: Regression analyses

Unadjusted analysis showed that piped water supply to houses and its consistency were associated with anaemia (daily water supplies OR 0.47, 95% CI: 0.28 to 0.80, p<0.001). Presence of toilets in the village decreased anaemia risk (OR 0.54, 95% CI: 0.37 to 0.78, p=0.001). Factors related with healthcare system such as access to Accredited Social Health Activist (ASHA) workers, government nurses, and private doctors were associated with a lower risk of anaemia (Table 6).

A fully adjusted analysis of village factors is outlined in the table 7. Piped water supply reported significant increase in Hb level (linear regression β coefficient 0.61, 95% CI: 0.39 to 0.82, p<0.001). In the fully adjusted logistic regression, piped water supplies and its regularity (p<0.001), toilet facilities (p<0.001), and access to government nurse (p=0.002) significantly associated with lower anaemia (OR 0.56, 95% CI: 0.35 to 0.90, p=0.01). Higher anaemia was seen in villages where government health facility was available (OR 4.77, 95% CI: 2.32 to 9.89, p<0.001).

Table 6: Characteristics of villages with unadjusted regression analyses (n= 34 villages)

Village Characteristics	Village (%)	Non-anaemic participants (%)	Anaemic participants (%)	Coefficient with 95% confidence interval	Linear regression p value	Odds ratio with 95% confidence interval	Logistic regression p value
Piped water supplied to houses							
No	10 (29.4)	14 (10.7)	263 (29.8)	Ref		Ref	
Yes	24 (70.5)	116 (89.2)	617 (70.1)	0.61 (0.42 to 0.79)	<0.001	0.28 (0.15 to 0.50)	<0.001
Daily water supply (at least once a day)							
No	7 (20.5)	18 (13.8)	222 (25.2)	Ref		Ref	
Yes	27 (79.4)	112 (86.1)	658 (74.7)	-0.04 (-0.23 to 0.15)	0.66	0.47 (0.28 to 0.80)	0.005
Operational community toilets							
No	23 (67.6)	64 (49.2)	565 (64.2)	Ref	-	Ref	-
Yes	11 (32.3)	66 (50.7)	315 (35.8)	0.12 (-0.04 to 0.29)	0.15	0.54 (0.37 to 0.78)	0.001
Government health centre availability (sub-centre/primary health centre)							
No	24 (70.5)	94 (72.3)	556 (63.1)	Ref	-	Ref	-
Yes	10 (29.4)	36 (27.6)	324 (36.8)	-0.09 (-0.26 to 0.07)	0.27	1.52 (1.01 to 2.28)	0.04
Accredited social health attendant (ASHA) appointed							
No	1 (3.0)	0	18 (2.0)	Ref	-	Omitted	-
Yes	33 (97.0)	130 (100)	862 (97.9)	0.88 (0.26 to 1.51)	0.006	-	-
Daily access to government nurse							
No	29 (85.2)	104 (80.0)	771 (87.6)	Ref		Ref	
Yes	5 (14.7)	26 (20.0)	109 (12.3)	0.00 (-0.23 to 0.25)	0.93	0.56 (0.35 to 0.90)	0.01
Government nurse conducts haemoglobin investigation							
No	8 (23.5)	32 (24.6)	171 (19.4)	Ref	-	Ref	-

Yes	26 (76.4)	98 (75.3)	709 (80.5)	-0.21 (-0.41 to -0.00)	0.04	1.35 (0.87 to 2.08)	0.17
Private doctor availability							
No	31 (91.1)	119 (91.5)	800 (90.9)	Ref	-	Ref	-
Yes	3 (8.8)	11 (8.4)	80 (9.0)	0.27 (-0.01 to 0.56)	0.06	1.08 (0.65 to 2.09)	0.81
Travel time to the nearest primary care hospital	Continuous variable	-	-	-0.00 (-0.01 to 0.00)	0.46	NA	NA

Table 6 footnotes:

- For linear regression, haemoglobin values as a continuous measure (was the main outcome) and for logistic regression anaemic or non-anaemic status (was the main outcome).
- Anaemia was defined as an Hb level below 12.0 g/dl.
- NA- Not applicable. Ref indicates reference category.

Table 7: Adjusted regression results: village level risk factors associated with anaemia in adolescent girls (N= 1,010 girls from 34 villages)

Characteristics	Unadjusted linear regression β (95% CI)	Fully adjusted model β (95% CI)	Unadjusted logistic regression OR (95% CI)	Fully adjusted model OR (95% CI)
Piped water supplied to houses				
No	Ref	Ref	Ref	Ref
Yes	0.61 (0.42 to 0.79)	0.61 (0.39 to 0.82)**	0.28 (0.15 to 0.50)	0.22 (0.12 to 0.41)**
Daily water supply (at least once a day)				
No	Ref	Ref	Ref	Ref
Yes	-0.04 (-0.23 to 0.15)	0.14 (-0.05 to 0.35)	0.47 (0.28 to 0.80)	0.27 (0.16 to 0.47)**
Operational community toilets				
No	Ref	Ref	Ref	Ref
Yes	0.12 (-0.04 to 0.29)	-0.06 (-0.26 to 0.13)	0.54 (0.37 to 0.78)	0.50 (0.32 to 0.77)**
Government health centre availability (sub-centre/primary health centre)				
No	Ref	Ref	Ref	Ref
Yes	-0.09 (-0.26 to 0.07)	-0.32 (-0.59 to -0.05)	1.52 (1.01 to 2.28)	4.77 (2.32 to 9.89)**
Accredited social health attendant (ASHA) appointed				
No	Ref	Ref	Ref	Ref
Yes	0.88 (0.26 to 1.51)	0.52 (-0.10 to 1.16)	Omitted	Omitted
Daily access to government nurse				
No	Ref	Ref	Ref	Ref
Yes	0.00 (-0.23 to 0.25)	0.15 (-0.12 to 0.44)	0.56 (0.35 to 0.90)	0.13 (0.05 to 0.29)**
Government nurse conducts haemoglobin investigation				
No	Ref	Ref	Ref	Ref
Yes	-0.21 (-0.41 to -0.00)	0.15 (-0.12 to 0.44)	1.35 (0.87 to 2.08)	1.19 (0.72 to 1.99)
Private doctor availability				
No	Ref	Ref	Ref	Ref

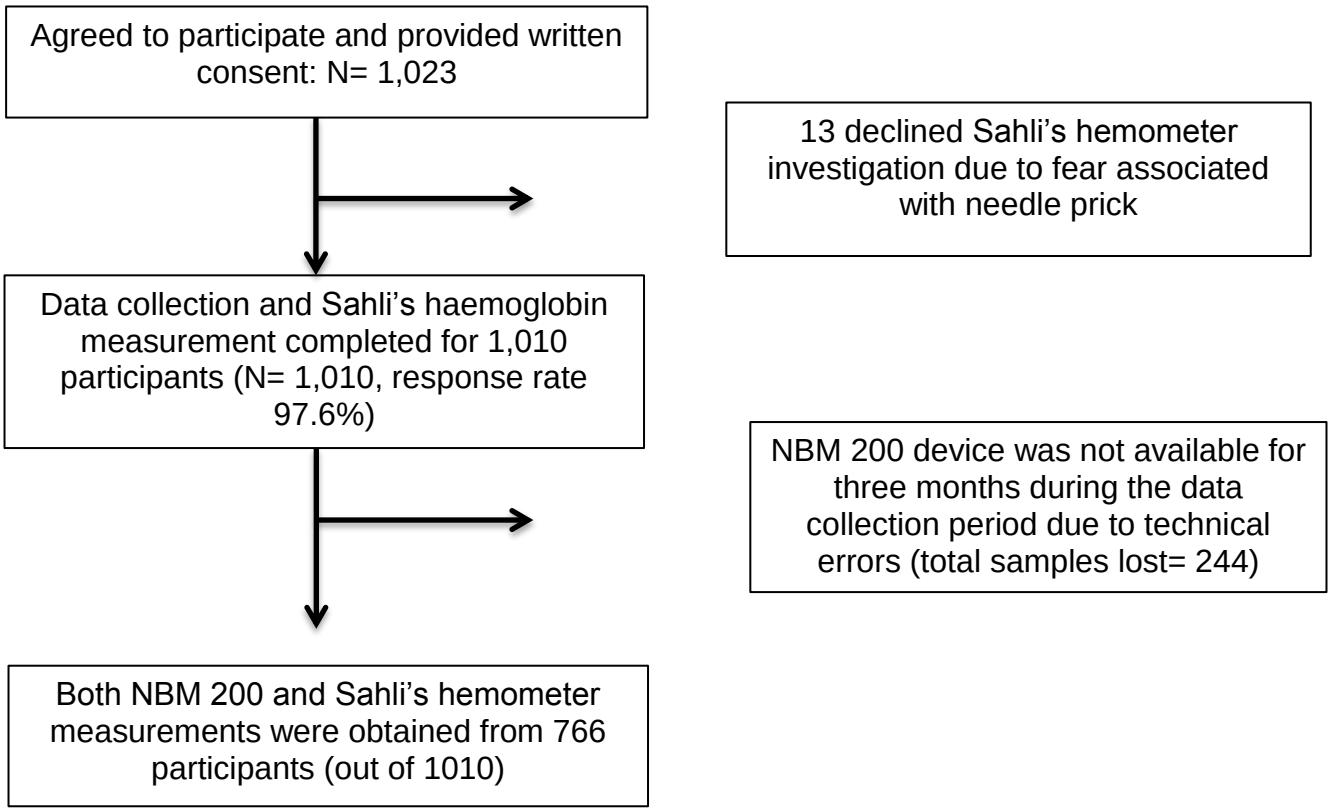
Yes	0.27 (-0.01 to 0.56)	0.20 (-0.13 to 0.54)	1.08 (0.65 to 2.09)	2.12 (0.96 to 4.67)
Travel time to the nearest primary care hospital (continuous variable)	-0.00 (-0.01 to 0.00)	-0.00 (-0.01 to 0.00)	NA	NA

Table 7 footnotes: *Indicates p<0.05, **Indicates p<0.001, NA- Not applicable. Ref indicates reference category.

3.4.4 Non-invasive haemoglobin technology comparison in adolescent girls

Due to technical errors reported by the NBM 200, the device was not available for three months during the study period. Thus, non-invasive Hb measurements were obtained from 766 participants (out of 1,010), and the same was used to conduct comparison with the Sahli's hemometer (Figure 14).

Figure 14: Flow chart of process implemented to obtain to obtain NBM 200 and Hemometer measurements

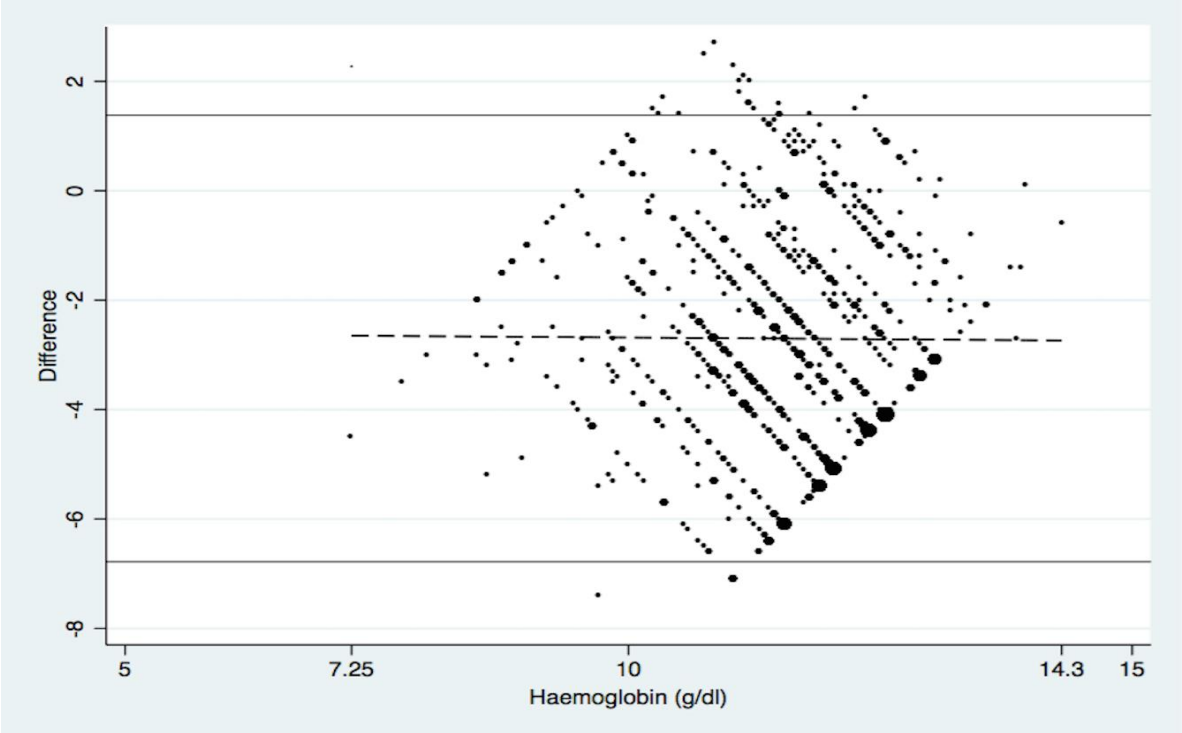


Haemoglobin estimated by Sahli's hemometer ranged from 5.0 g/dl to 14.0 g/dl with a mean of 10.1 g/dl (SD: 1.41). While the non-invasive sensor (NBM 200) yielded Hb estimates in the range of 9.5 g/dl to 15.2 g/dl (SD: 1.42) with a mean Hb of 12.8 g/dl. The reference method (Sahli's hemometer) showed a high prevalence of anaemia

(84%) while, the NBM 200 diagnosed only 26% of participants (out of 766) as anaemic. The hemometer identified 41 participants (5%) with severe anaemia (Hb 7.9 g/dl and below), and the NBM 200 sensor did not identify any severe anaemic participants.

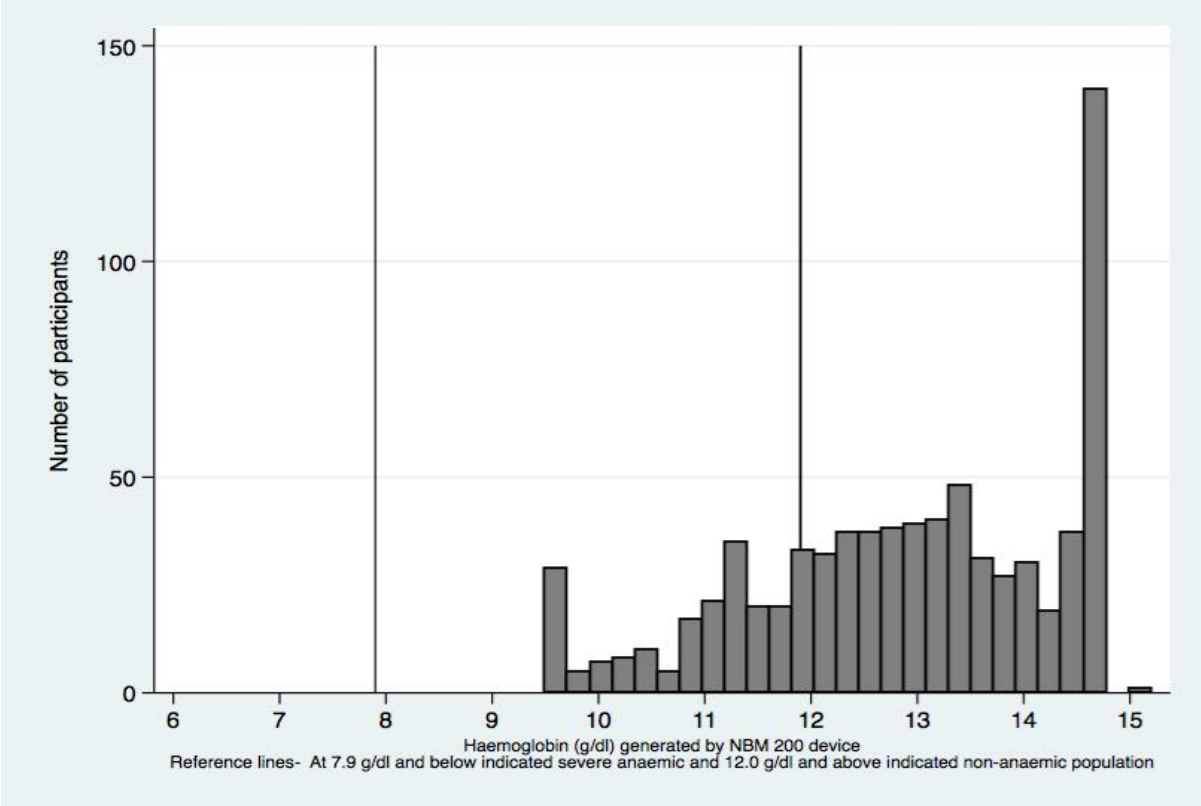
The Bland-Altman analysis showed a mean difference of -2.70 g/dl (95% CI: -2.84 to -2.55) in Hb estimates suggesting a systematic overestimation of haemoglobin using the non-invasive technique (Figure 15). The Bland-Altman plot showed a diamond shaped pattern suggesting that there are approximately 15 subgroups with a linear association between Sahli's hemometer measurements and the difference between the two devices. Examination of this in more detail showed that this is a consequence of the difference between the two measurement techniques being similar for the number of readings.

Figure 15: Bland Altman Plot of Sahli's Hemometer and NBM 200 (N=766)



A histogram plotting Hb estimates obtained using Sahli's method (reference method) showed a normal distribution (page number 76), while the histogram of Hb estimates obtained using the NBM 200 was not normally distributed, and showed plateau like presentation (Figure 16).

Figure 16: Histogram of Hb measurements obtained using NBM 200 (N=766)



A statistically significant weak negative correlation was found between the two methods (Spearman's test rs = -0.07, p= 0.03). The NBM 200 had low sensitivity (23.6%, 95% CI: 20.4 to 27.1), moderate specificity (61.8%, 95% CI: 52.6 to 70.4), low negative predictive value (13.4%, 95% CI: 10.7 to 16.5) and moderate positive predictive value (76.4%, 95% CI: 69.9 to 82.1). The AUROC score was 0.43 suggesting poor anaemia diagnostic performance of the NBM 200 in our study population.

Sensitivity analysis of a subgroup of data collection over two moths (April to May) resulted in a mean difference of -0.51 g/dl with wide limits of agreement (-3.46 g/dl to 2.43 g/dl), demonstrating a consistent overestimation of Hb measurement by NBM 200 compared to the Sahli's hemometer.

3.5 Discussion

3.5.1 Principal findings

The prevalence of anaemia in adolescent girls in our study area of rural Maharashtra was extremely high (87%) with the majority of participants having moderate anaemia (65%). Higher risks of anaemia were recorded with increasing age, low fruits intake and low MUAC (< 22 cm). While village factors such as consistent piped water supplies, and access to healthcare providers were associated with anaemia. Thirdly, the non-invasive technology (NBM 200) underestimated anaemia prevalence (sensitivity 23.6%), and failed to detect severe anaemia cases in our study population, when compared with hemometer estimates.

3.5.2 Strengths and limitations

To our knowledge, this is the largest study from Maharashtra state where findings from a large rural adolescent population are presented. The response rate of 97.6% was high, thus minimising bias in the data collected and also improved generalisability. None of the study participants had thumb injury, ulceration or any skin colourants (such as Henna) thus allowing for Hb measurements to be obtained in accordance with the guidelines [79]. We also conducted regular quality controls to

ensure optimal data quality, such as periodic equipment checks as outlined earlier in the methods (page number 49).

It is important to note that this analysis has a number of limitations. Study limitations are outlined below followed by explanation on each limitation.

- Sample size and chance
- Generalisability
- Residual confounding
- Bias in data collection
- Measurement error
- Reverse causality
- Limitations related with blood investigations

Sample size and chance: This was a feasibility study due to the limited timeframe and financial resources available for data collection, however, our sample of 1010 adolescent girls was the largest study assessing anaemia prevalence in rural Maharashtra. We also did not have a good estimate of the anaemia prevalence as this ranges considerably across India.

Generalisability: The study observations are applicable to Maharashtra State, and not necessarily generalisable to other states in India. It was not possible to compare our estimates to any baseline population data on anaemia, as there is no information recorded in this rural population.

Residual confounding: We were assessing a number of risk factors for anaemia in this section and whilst covariates were mutually adjusted, detailed statistical assessment of confounding was not undertaken. It is important to note that complete

and accurate information on certain factors was lacking, in particular for diet, as the dietary responses questionnaire will have obtained a summary of approximate diet, and will be subject to recall. Also, while an attempt was made to collect data on all possible relevant covariates based on published literature, it is possible that there may be other unknown factors in our study population that are associated with adolescent anaemia.

Bias in data collection: Information on dietary intake, anaemia history, and menstrual health related variables are subject to bias, and this may increase the lack of precision of the estimates, as it is likely to be a non-differential bias. Information on other variables collected may have been affected by reporting bias such as variables related with socioeconomic status and household resources. However, every effort was made to verify such information with the HMF's village healthcare workers at the time of data collection. Subsequently, information on health and social data were collected prior to anaemia investigation, when adolescent girls, and also data collectors were not aware about the anaemia status. Thus, our study is less likely to have any differential bias. Though every attempt was made to minimise biases in our study design, some residual biases are inevitable in observational epidemiological studies.

Measurement error: The Hb measurement technique (Sahli's Hemometer) has a subjective element in the interpretation of the reading, and thus non-differential measurement error may exist in our Hb readings.

Reverse causality: The fully adjusted results showed an association between low mid-upper arm circumference (MUAC) and anaemia, which may be a true association, or possibly a phenomenon of reverse causality. MUAC is an indicator of nourishment, and malnourished individuals may have less opportunity to earn

money, thus further increasing the risk of malnutrition in a ‘vicious circle’ of deterioration. Therefore, chronic anaemia may lead to low MUAC due to combination of deficiencies. Because of our cross-sectional study design, further investigation of this relationship was not possible.

It is unlikely that there was reverse causation related to other risk factors measured.

Limitations related with blood investigations: Venous blood withdrawal in adolescent population was not feasible due to the logistic challenges of venous blood sample transport of more than 1,000 tests from field sites to the laboratory mainly because of intermittent electricity supply and poor road infrastructure. It is important to note that venous blood withdrawal is not routinely used for anaemia screening in the adolescent population in our field area, and insisting on this for blood investigation using an automated analyser, may have adversely affected our study response rate. Secondly, there are many causes of anaemia including iron, vitamin B12 or folic acid deficiency, which requires advanced blood investigations, includes serum iron, folic acid, and vitamin assessments. However, due to absence of micronutrient assay facilities at HMF Hospital coupled with limited funding, these investigations were not conducted in the study. Thirdly, the Bland-Altman plot (Figure 15) formed a diamond shaped pattern suggesting that the difference between two Hb measurement techniques was highly variable. The pattern is likely due to technique variability (which could affect measurements obtained from either the NBM 200 or Sahli’s hemometer). Such technique variability may occur due to variation in daylight, subjectivity related with either the hemometer or the technician. The variability was quite apparent for frequently occurring Hb values (for e.g. 94 participants had an Hb value of 9.5 g/dl), and less apparent for rarer Hb values, resulting in the diamond shaped plot.

3.5.3 Synthesis

Individual and village level risk factors of anaemia

Interpretation from Individual risk factors

The primary analysis showed associated risk factors, and also provided foundation to develop the multivariable modelling strategy. Covariates such as knowledge about anaemia, past Hb investigations were not included in the modelling due to recall biases. Below poverty line registration (BPL) data were validated against family resources (land, vehicles, TV), which showed some discrepancies, thus excluded from the adjusted analysis [80]. Recent studies published on BPL registration and health services suggested that those who were in need of support are less likely to hold the registration due to administration difficulties in rural areas where government subsidises are often secured by affluent families [17,80,81]. Agriculture is the main source of income in the study area where 80% families are engaged in farming, thus size of the land and house structure were considered to be an appropriate marker of socioeconomic status following consultation with HMF.

The multivariable analysis showed that risk of anaemia was higher with increasing age, which is consistent with prior studies from Maharashtra [52,62], and some of this increase in risks could be due to menarche [48,70]. Studies show that low haemoglobin levels are associated with delayed menarche, while high menstrual flow is a direct cause of low haemoglobin [82]. However, we did not find any difference between pre and post-puberty participants (p=0.27).

Low fruit consumption was associated with a higher risk of anaemia. Common iron rich fruits consumed in our study population were apples, grapes, watermelon and pomegranate, which have high iron content. A recent clinical trial showed an increase in

iron levels as a result of high fruit consumption, particularly due to vitamin C, which improves iron absorption [83]; it is therefore, likely that those who consumed more fruits were more likely to have increased iron absorption resulting in lower risk of anaemia.

Low MUAC (an indicator of poor nutrition) was associated with an increased risk of anaemia, and it is recognised that an overall compromised nutritional status is likely to affect haemoglobin levels [71,84]. Our findings showed higher Hb levels with increased rice consumption, which may be due to the type of rice, and the way it was consumed in our study population; for example, combination of rice with other food items such as traditional Indian curries (generally made up from range of vegetables) may influence haemoglobin levels [85]. Secondly, unpolished rice (brown rice) was commonly consumed in the study area, which has higher iron content than the polished rice (brown rice: 5.8 mg of iron/100 gram) [86]. The high prevalence of both anaemia (87%) and low BMI (67%) may be the consequences of chronic malnutrition influenced by food availability, costs and preferences [84,87].

We observed higher anaemia in those who were on IFA supplements; 99% of those attended school, of whom 75% had undertaken an Hb investigation in the preceding 6 weeks at school, following which they were included in the school-based weekly supplementation programme. In total only 93 girls (of 1,010 participants) were consuming IFA supplements at the time of data collection. The majority of these individuals (73 girls of 93) started receiving weekly supplements in the same month that data were collected, which could explain the association with anaemia (supplementation initiative started by the school with not enough time elapsed for the supplements to have had an observable impact on Hb levels). National anaemia guidelines suggest that a minimum of 3 months

of daily iron supplementation is required to reverse anaemia in the absence of any major underlying pathology [68,74].

Less anaemia was observed in school dropouts in our study population, 42% of whom were engaged in agricultural employment. This may be explained by the fact that employment in our field area typically provides remuneration on a weekly basis (an average 10-15 GBP per week for 6 days of work for adolescents), which may have provided opportunities to purchase additional food resources. Based on our understanding of the field area, we hypothesise that this small earning by young girls was likely to support the purchase of supplementary food items. As a result, the improved access to food items may have improved Hb levels. It is important to note that our analysis showed no association with menarche, socioeconomic status and family size in our study population.

Interpretation of village level risk factors

Our findings showed that regular piped water supplies, and toilet facilities were associated with lower risks of anaemia. We understand that the following two main factors may play a major role in our study population, a) water security, and b) personal hygiene/sanitation.

Water security is a major concern in Marathwada region, where rural areas face draught situations every three to five years [88]. Water availability varies in the field area, and there are no centralised regulated supplies. Depending on the availability, village authorities stock and provide piped water to individual houses, and in case of a shortage, water is stored at communal place, from where, it is collected and

transported by individual households. In the MAS study area, and also in the Indian society, girls support their mother in household tasks, which include securing water for daily usage. We understand that young girls are often needed to travel on foot to collect water, for both drinking and utility purposes (Appendix 2 photographs). The process is tiring, and also demands more food intake. We observed that about 30% of study participants belong to poor families with limited resources to support nutrition requirements. Therefore, villages where piped water supplies was guaranteed may have provided better opportunities to improve health status compared to those where supplies were less reliable.

Secondly access to sanitation facilities (including community/personal toilets) improves public health [89]. A recent analysis from National Family Health Survey of India (NFHS 3) showed that toilet facilities and improved personal hygiene reduced anaemia in under five year old children [90]. Access to water promotes frequent hand washing, and also improves hygiene by reducing chances of infections (such as soil-transmitted helminthiasis), which directly lowers anaemia risks [91]. Further investigation using the MAS data showed that 116 non-anaemic participants (90% of the total non-anaemic population, N = 130) had regular piped water supplies compared to anaemic girls where water needs to be collected from public places for daily usage ($p<0.001$). Similarly 50% of non-anaemic participants in our study had access to toilet facilities reducing risks of anaemia ($p= 0.004$).

Our findings indicated that routine access to healthcare providers was associated with a lower risk of anaemia. We understand that healthcare access guaranteed periodic free Hb investigations. Of 130 non-anaemic participants, 80% (104) had

daily access to a government nurse, which may have provided opportunities to seek medical assistance.

On contrary higher risks of anaemia were seen in villages with health centre facilities. Ten villages (of 34) had government health centres, where Hb investigation facility was available. None of the centres were involved in IFA supplementation. We understand that this association is due to access to health centre where Hb investigation was performed but no further medical supplementation was available, thus data reported higher risks in villages with health centre facility. A study on government IFA supplementation in rural areas of Ahmednagar district of Maharashtra showed that three hospitals (of 14) did not receive medicine supplies in one whole calendar year [92], similar to the MAS project area. However, we did not collect information on iron supplies to government hospitals during the study period, thus the association cannot be further scrutinised. Thus, such finding should be interpreted with caution.

Based on our field observations and consultations with the local partner (HMF), we recognise that community resources including infrastructure and healthcare seem important in adolescent health. It is vital to understand anaemia from comprehensive public health perspectives, where individual medical conditions and also the environment need to be further examined to identify reasons of high prevalence in the country.

3.5.4 NBM 200 device assessment

We acknowledge that the Sahli's hemometer is not a gold standard technique to measure haemoglobin. Though we have used the hemometer as the reference, our

results are in agreement with another study (part of this MAS project) evaluating the NBM 200 with the automated haematology analyser (Sysmex XP-100) in pregnant women in rural Maharashtra (N=269) [93]. Results from the pregnant women study showed that the NBM 200 overestimated haemoglobin levels with subsequent underestimation of anaemia status (sensitivity of 33.7%) when compared with venous Hb measurements (detailed in chapter 4). Findings further reported a mean difference of -1.8 g/dl (95% CI: -2.06 to -1.71), which is in agreement with this adolescent girl analysis.

To our knowledge, there is no published evidence comparing the NBM 200 with Sahli's hemometer. A study has compared another finger prick technique known as HemoCue (ANGELHOLM, Sweden) with NBM 200 in a blood donation centre [94]. The HemoCue uses peripheral capillary blood similar to the Sahli's hemometer, but is considered to have a better precision because of reduced human error. However, Kim et al stated that when HemoCue and NBM 200 were compared to an automated haematology analyser (LH500, Beckman Coulter Inc., Brea, USA), both (NBM 200 and HemoCue) showed low sensitivity (38% and 47% respectively) [94]. We acknowledge that Sahli's hemometer estimates are susceptible to interpretation. However, given that this is the most commonly used technique in India, comparison of the two techniques was useful in a rural setting to understand NBM 200 applications. Hemometer is used very widely in Indian rural locations mainly due to low operational costs, portability and also the ability to conduct investigation without electricity or technical support, which is often required for, automated analysers and also for HemoCue.

As stated earlier, the NBM 200 failed to detect severe anaemia in our study population [93]. The technology is validated in western population which has different skin tone/type compared to Indian setting, which could be the reason of the failure. However, this needs further investigations. Based on our findings, the NBM 200 cannot be used as an alternative to the current anaemia diagnostic technique. If improved substantially or re-calibrated by manufacturers in near future to provide precise measurements in an Indian setting, non-invasive technology may be useful for anaemia screening to facilitate early diagnosis and referrals.

3.5.5 Conclusion

Anaemia prevalence was extremely high among adolescent girls in rural areas of Maharashtra. Older age, low mid upper arm circumference (typically less than 22 cm), and low consumption of fruits were major risk factors resulting in anaemia among adolescent girls. At village level important infrastructure related concerns primarily water supplies and access to healthcare providers were modifiable risk factors of anaemia. Future research is required to identify reversible causes of anaemia in rural India, and test the hypotheses generated.

This research is the first to provide a comparison between Sahli's hemometer and the NBM 200 in developing country setting. We report a systematic overestimation of haemoglobin using the NBM 200, which is consistent with our study involving pregnant women in the same geographical location in India. There is an urgent need to improve the non-invasive technology, as it will provide opportunities in resource poor areas for mass screening where high prevalence of anaemia is known.

Chapter 4. Pregnant women: Prospective study

4.1 Introduction

This chapter describes the second component of the MAS (Pregnant Women Study). Broadly the chapter has four parts; literature review, methods, results and discussion. Literature review talks about anaemia in pregnancy in the Indian population, current research gaps, and outlines the study purpose. The method section elaborates study objectives, blood investigations and statistical analysis. The results comprise of individual and village level risk factors of anaemia, risk factors of low birth weight, and the last section reports on the non-invasive haemoglobin technology validation (NBM 200). Discussion has main findings from the study, strengths, limitations followed by synthesis. Manuscripts developed from pregnant women data are mentioned in the appendix 12 (list of publications).

4.2 Literature review

4.2.1 Studies from India and Maharashtra on maternal anaemia and birth weight

Iron deficiency anaemia (IDA) is a very common form of anaemia observed in pregnancy [95]. Findings from the recent Indian National Family Health Survey (NFHS 3, 2005-06) reported that 56% of women (between 15 to 49 years) were anaemic, of whom 60% were from rural areas [13]. The high prevalence of anaemia in rural areas was mainly observed due to a lack of medical infrastructure to diagnose and treat anaemia [18]. Furthermore, interrupted medicine supplies (of iron folic acid) restricted opportunities to reduce anaemia prevalence [92].

Studies from India reported the prevalence between 17% and 89% (table 8). Such variation is likely to exist due to study setting (hospital/community), territory (urban/rural), and also due to variations in dietary preferences/resources [13]. For example, studies from South India showed lower prevalence compared to studies conducted in north India. Such difference may subsist due to improved healthcare access in southern states compared to northern territories [13]. Data from national surveys (conducted in 1996 and 2005) were used to investigate prevalence of low birth weight (LBW). The analysis reported 23% of LBW prevalence in 1996 and 20% in 2005 [13] .

The majority of the regional studies (outlined in the table 8) were conducted in hospital environments, and very limited evidence is available from community-based research. Possible reasons are challenges in identifying and recruiting pregnant women from communities. Furthermore, access to rural communities is difficult due to limited transport, poor health and social infrastructure, which are likely to be the reasons for lack of evidence from rural areas of the country. Risk factor analyses in the studies outlined in table 8 included demographic aspects such as maternal age, BMI, religion/caste, education and parity. None of the currently published literature evaluated village level risk factors associated with anaemia during pregnancy, which may have a role mainly in rural populations.

Information on maternal anaemia and low birth weight from Maharashtra state is available. However the majority of studies (table 9) were cross sectional surveys conducted in the districts located in western Maharashtra (such as Pune, Sangli, Nasik), which has higher per capita income than the Marathwada region [26].

Results from a hospital based case control study conducted in Sangli city (N=139) showed 23% of prevalence [96]. Analysis from other areas of Maharashtra (Sholapur, Nasik and Latur cities) stated a prevalence from 30% to 50% [97,98].

To our knowledge, there are only two community cohort studies published from Maharashtra state [99]. The first study to identify prevalence and determinants of low birth weight (LBW) was conducted in rural areas of Pune district in 1994 (45 villages) [99]. The Pune study showed an increased risk of LBW with i) maternal haemoglobin (Hb) less than 9.0 g/dl, ii) third trimestral bleeding, and iii) low maternal body mass index (BMI). However, risks of maternal anaemia based on Hb levels were not assessed in the study.

A second cohort study published in 2010 from Pune district showed 77% prevalence and reported an association between younger age at marriage and maternal anaemia [100]. Nevertheless this study by Rao et al considered limited factors in the risk analysis namely age at marriage, age at menarche, education, parity, milk/green leafy vegetables/fruits consumption. Furthermore, the study was conducted only in three villages located near Pune city. At national level, surveys were conducted to estimate prevalence of maternal anaemia and low birth weight as outlined earlier, however to our knowledge no cohort studies were performed.

Therefore, currently limited data is available from Maharashtra state and also from India on maternal anaemia during pregnancy and its effect on birth outcomes.

4.2.2 Survey purpose

National Family Health Surveys (NFHS) involved the population from Maharashtra state, where 48% anaemia prevalence is recorded in the recent survey (NFHS-3, 2005-06) [101]. However none of the studies outlined earlier including the NFHS conducted detailed risk factor assessment to investigate high prevalence during pregnancy. To our knowledge, limited evidence is available on this important public health issue, particularly in the rural region of Maharashtra (such as Marathwada). Furthermore, there is no community based research from Marathwada region (approximately 18 million population), which is one of the most marginalised areas of the state and the country [27].

Secondly, none of the published literature studied village level risk factors associated with maternal anaemia, which is an important area in understanding the health issue comprehensively. The village or community setting includes healthcare access, sanitation, transport facilities which play important role in public health systems, thus it needs to be considered to investigate the long standing high anaemia prevalence in the country.

Therefore, we planned a prospective study involving pregnant women from rural areas to estimate the prevalence and risk factors for anaemia during early stages of pregnancy. The study also investigated prevalence and risk factors associated with low birth weight.

Table 8: Published studies involving pregnant women in India, prevalence and risk factor for maternal anaemia and low birth weight (10 studies)

Author, publication year and study type	Study location in India (setting and state)	Sample size, anaemia cut off	Risk factors of maternal anaemia/LBW	Anaemia/LBW prevalence	Additional information/analysis
Rohilla et al 2010 [102], Survey	Hospital based study in Chandigarh city, North India	N=4456 pregnant women (Hb cut off 11.0 g/dl)	None	Of 4456 women, 798 were anaemic (17.9%) includes 96 women with severe anaemia (2.1%).	Complications of severe anaemia- Preterm labour and premature rupture of membrane were the most common causes. No regression analysis.
Bharati et al 2008 [103], Survey	NFHS-2 data from 28 Indian states	N=5619 pregnant women (Hb cut off 11.0 g/dl)	Maternal age, religion, ethnicity, education, BMI.	47.5% prevalence (28 states). Maharashtra state prevalence 51.8%	Only linear regression results were presented. No adjusted analysis.
Bora et al 2014 [104], Survey	Hospital based study in Dibrugarh, Assam, North east India	N=470 pregnant women (Hb cut off 11.0 g/dl)	None	89.6% prevalence.	Neonatal outcomes based on severity of anaemia. Risk factors of LBW- mild and severe anaemic women compared to non-anaemic group.
Abel et al 2001 [105], Survey	Rural area, Vellore district, South India	N=845 women in total from three trimesters, (Hb cut off 11.0 g/dl)	None	1st trimester-56.1% 2nd trimester- 73.4% 3rd trimester- 68.8%	Blood parameters during pregnancy- haemoglobin, serum iron levels
Balarajan et al 2013 [106], Survey	NFHS-2 and NFHS-3 data (all	N= 22,648 births from both NFHS	Risk factor of low birth weight- IFA,	LBW prevalence in 1998/99- 23.1%	Risk factors of LBW- No association with IFA and

	Indian states and territories)	surveys, Cut off not mentioned	ANC, maternal age, parity, mother's education, caste, mother's BMI, socioeconomic status	In 2005/06- 20.5%	antenatal care. Regression with adjusted analysis.
Mavalankar et al 1992 [107], Case control study	Hospital based study Ahmedabad city, West India	Cases-1317 Control- 1465 Cut off not mentioned	Risk factor of LBW- low maternal weight, poor obstetric care, lack of ANC, maternal anaemia, hypertension, increase exposure to cooking smoke	LBW prevalence- 30%	Risk factors of LBW- Low maternal weight, poor obstetric care, lack of antenatal care, maternal anaemia, hypertension were associated with LBW. No adjusted analysis.
Malhotra et al 2014 [108], Survey	NFHS-3 data (all Indian states and territories)	Not clear, Cut off not mentioned	Risk factor of LBW- Dietary intake, IFA supplementation, maternal height, weight, religion	LBW prevalence- 22%	Risk factors of LBW- IFA supplementation, dietary intake of milk, fruits, fish decreased risk of LBW baby. No adjusted analysis.
Perumal 2014 [109], Survey	NFHS-3 data (all Indian states and territories)	N= 3355 from rural areas, and N= 1962 from urban areas. Cut off not mentioned	Risk factor of anaemia- wealth index, maternal education, BMI, current children,	Anaemia prevalence Rural area- 32.4% Urban area- 27.3%	Risk factor of anaemia- in rural areas Increasing age, mass media exposure protective factor for anaemia.

			mass media, tobacco exposure		In urban areas Low maternal weight risk factor for anaemia. Regression with adjusted analysis.
Ganesh Kumar et al 2010 [40], Case control study	Hospital based study in Mangalore city, Karnataka, South India	Cases- 150 Control- 150 Cut off not mentioned	Risk factors of LBW- maternal age, parity, ANC, maternal height, weight, anaemia status,	Anaemia prevalence- 36%	Risk factors of LBW- Maternal age. Regression with adjusted analysis.
Noronha et al 2010 [110], Cohort study	Three towns in Udupi district, Karnataka, South India	N= 1077 for pre-delivery data and N=1000 for post-delivery analysis (Hb cut off 11.0 g/dl)	Risk factor for anaemia- maternal age, education, parity, spacing, BMI, food selection ability	Anaemia prevalence- 50.14% (pre-delivery data) and 53.7% in post-delivery analysis	Risk factor of anaemia- low socioeconomic status. Regression with adjusted analysis.

Table 9: Published studies involving pregnant women in Maharashtra state, prevalence and risk factor for maternal anaemia and low birth weight (6 studies)

Author, publication year and study type	**Study location in Maharashtra (city/district)**	**Sample size, maternal anaemia Hb cut off**	**Risk factors of maternal anaemia/LBW**	**Anaemia/LBW prevalence**	**Additional information/analysis**
Hirve et al 1994 [99], Cohort study	Rural area, Pune district (45 villages)	Not clear (approximately 1922), Hb cut off for maternal anaemia was not mentioned	LBW- Maternal BMI, Hb, Bleeding during third trimester	LBW prevalence- 29%	Risk factors of LBW- None of the factors reported association in the adjusted analysis.
Kinare et al 2000 [111], Cohort study	Rural area, Pune district (6 villages)	Pregnant women N= 487, Birth weight analysis N=391, (Hb cut off 11.0 g/dl)	None	Not mentioned	Placental volume and foetal growth parameters (includes birth weight)
Rao et al 2011 [100], Survey	Rural area, Pune district (3 villages)	Not clear (varying sample size for each analysis in the study, total study population n=418), (Hb cut off 11.0 g/dl)	Anaemia- Age at marriage, age at menarche, education, parity, Milk/Green leafy vegetables/fruits consumption	Anaemia: 77% women had anaemia (Hb <11.0 g/dl) includes 59% mild anaemia and 16% moderate anaemia, 2.2% had severe anaemia	Risk factor of anaemia- Age at marriage reported significant association with risk of iron deficiency anaemia (regression analysis).

Mumbare et al 2012 [97], Case control study	Hospital data from Latur and Nasik cities	274 cases and 274 controls, Hb cut off was not mentioned	LBW- Maternal BMI, gravidity, previous children, tobacco, maternal anaemia.	Of 274 cases, 52.2% had anaemia.	Risk factors of LBW- maternal height, weight, birth space hypertension, inadequate ANC care (no adjusted analysis performed).
Haralkar et al 2013 [98], Survey	Rural area, Sholapur district (1 village)	Mixed population of pregnant and non-pregnant women (N=529), (Hb cut off 11.0 g/dl)	Anaemia- Age, family size, education, age at marriage	Of 529, 27.6% were anaemic (includes both pregnant and non-pregnant women, independent proportions are not mentioned).	Risk factor of anaemia- age, education, regression analysis (no adjusted analysis performed).
Mahavarkar et al 2008 [96], Case control study	Hospital data from Sangali city	Cases- 139, controls-530 (Hb cut off 10.5 g/dl)	LBW- Preterm labour, anaemia, hypertension, mode of delivery.	Prevalence Cases- 23.05% Control- 10.8%	Risk factors of LBW- regression analysis and adjusted analysis

4.3 Methods

4.3.1 Study objectives and eligibility

The MAS aimed to investigate the prevalence, individual and village level risk factors associated with anaemia, among women who were 3 to 5 months pregnant. The study also assessed risk factors of low birth weight. MAS field area consisted of 35 villages (approximately 65,000 population) from Tuljapur and Lohara blocks of Osmanabad district (Appendix 1 study village list). Details of eligible pregnancy identification and data collection are outlined in chapter 2 (page number 42 to 46).

Recruitment was based on the two main criteria:

1. Resident of the study area
2. Confirmed pregnancy of 3 to 5 months identified during the recruitment period (24th April 2014 to 31st December 2014, 252 days).

Women were eligible to participate from the start of a 3rd month of pregnancy until the end of 5th month (8 weeks to 20 weeks). The total study duration was of 433 days (24th April 2014 to 30th June 2015), including the post-delivery data collection. The recruitment was permitted until 31st December 2014 in order to collect post-delivery data on pregnancy outcomes by the outlined June 2015 deadline.

The secondary objective of the study was to validate the NBM 200 technology in the Indian pregnant woman population. Haemoglobin measurements obtained from the NBM 200 were analysed against the Hb measurements generated by an automated haematology analyser (Sysmex XP-100, Sysmex Corporation, Japan).

4.3.2 Data collection

The primary investigator (PI) with the help of an assistant and village health workers (VHWs) approached eligible pregnant women in the field area. The field based data collection process is outlined in the main methods (page number 42 to 46).

Two levels of data were collected: Individual participant data, and information on the village/community infrastructure.

4.3.3 Individual participant data

Data were collected twice from each participant, once during pregnancy, and a second time after delivery. The individual datasheet had sections on sociodemographic characteristics, medical/obstetrics history, iron/calcium supplements, vaccinations, dietary preference, 7-days diet recall, family assets, antenatal services, and pregnancy outcomes as self-reported by participants, in accordance with the Standard Operating Procedures (SOP) [112].

Pre-delivery data collection

Pregnant women were identified through HMF's healthcare workers via monthly household surveys of women in the reproductive age group (15 to 49 years), who were residents of the study villages (page number 42 to 46). No formal sample size calculation was conducted, as the project was a feasibility study to identify future anaemia research priorities.

Anthropometric measurements included height, mid upper arm circumference (MUAC) of the dominant hand, and were recorded using measuring tapes. Weight was

ascertained using a digital weighing machine (OMRON Healthcare). Blood pressure (BP) was measured using the right hand in a sitting position with an automated digital BP monitor following the manufacturer's recommendations (OMRON Healthcare). Details of blood investigations are outlined below.

Blood investigations and anaemia assessment

Each participant was subjected to a single blood withdrawal. Venous blood withdrawal was performed in a supine position using the non-dominant hand by a qualified technician with a 2 ml sterile disposable syringe. The blood sample was transferred into two separate sterile tubes (1 ml each); one with Ethylenediaminetetraacetic acid (EDTA) for Complete Blood Count (CBC) investigation, and the second, to a tube containing fluoride solution for random blood sugar (RBS) estimation. Both samples were carried in a blood transport box to the HMF hospital laboratory, and tested within the first four hours of withdrawal for each participant. The CBC test was performed using an automated haematology analyser Sysmex XP-100 (Sysmex Corporation, Japan). Serum glucose was estimated using Erba Chem Touch semi-automated analyser (Erba Mannheim, Germany). Both machines were calibrated everyday according to the manufacture's guidelines, and were inspected every two months by an engineer for quality assurance and performance purposes. Details on data quality are outlined earlier (page number 49).

Anaemia in pregnancy was defined as a haemoglobin level of less than 11.0 g/dl (10.9 g/dl and below) as recommended by the Government of India [113]. The same classification for pregnant women is advised by the World Health Organisation [1]. Further

classification into mild, moderate and severe anaemia was made based on haemoglobin level to facilitate post-investigation referral [113].

a. Severe anaemia when Hb is less than 7.0 g/dl (6.9 g/dl and below)
b. Moderate anaemia when Hb is between 7.0 g/dl and 9.9 g/dl
c. Mild anaemia when Hb is between 10.0 g/dl and 10.9 g/dl
d. Participant were labelled non-anaemic when Hb is 11.0 g/dl and above

Low birth weight (LBW) was defined as less than 2.5 kilograms of weight (< 2500 grams) at birth [6]. Diabetes was defined when RBS level was ≥ 200 mg [114]. Any pathological changes in white blood cells, platelets and red blood cells were recorded if reported by the Sysmex XP-100 automated analyser. Hypertension was diagnosed if the average of two consecutive measurements were more than 140/90 mm of Hg [115].

All participants were informed about investigation results by telephone within 24 hours of blood withdrawal, and those with severe anaemia were urgently referred to participants' preferred hospital. Participants were also provided with hard copies of blood investigation reports within 72 hours from the day of recruitment [112]. The report included findings from anthropometric measurements, CBC and RBS investigation results (Appendix 7).

Non-invasive Hb measurement technology

Details on the NBM 200 technology, scope and application are outlined earlier in the chapter 3 (page number 67). NBM 200 measurements were obtained at field level after the blood withdrawal (in the same visit), and were recorded on data forms. Hb measurements acquired from the NBM 200 were used for analysis purpose only, and

were not provided to participants. Hb measurements by the Sysmex XP-100 were considered as the reference values for the NBM 200 validation. The Sysmex XP-100 being an automated venous blood analyser is considered as the gold standard for the non-invasive technology assessment [75,93].

Post-delivery data

All participants were contacted twice during their pregnancy term to inquire about their overall wellbeing. Data on post-delivery outcomes were collected over telephone as explained earlier in detail (page number 42 to 46).

4.3.4 Village profile data

Village profile had information on village infrastructure as outlined in chapter 3 (page number 46). These data were used to assess village level risk factors associated with anaemia.

4.3.5 Statistical analysis

Data were cleaned and checked wherever required. Analyses were performed using Stata 13.1 (StataCorp, College Station, Texas, USA). Initially frequencies and proportions were calculated. Linearity of variables such as age, gravidity, and number of children were assessed wherever necessary, before considering the variable, as a continuous or categorical measure. In all analyses statistical significance was defined as p value less than 0.05.

Analysis was grouped in the four sections:

1. Describing the prevalence and severity of anaemia in pregnancy

2. Risk factors associated with anaemia- both individual and village level risk factors were analysed.

3. Risk factors associated with low birth weight- individual level risk factors were analysed.

4. Validation of non-invasive haemoglobin technology (NBM 200) by comparing with the venous Hb measurement (Sysmex XP-100).

1. Prevalence and severity of anaemia

Those with Hb less than 11.0 g/dl were identified as anaemic, and the prevalence was calculated in the studied population. Further categorisation in mild, moderate and severe was performed as outlined previously to refer participants to the appropriate health facility.

2. Risk factors associated with maternal anaemia

Individual risk factors: Regression techniques were used to investigate risks associated with anaemia during pregnancy [76]. Linear regression was used to assess the association between each risk factor with haemoglobin level, and results were presented in regression coefficients (β) with 95% confidence intervals (CI). Logistic regression was used to estimate odds ratios (OR) and 95% CI for the association of each risk factor with anaemia status (binary categorical variable: anaemic/non-anaemic). For both regression techniques unadjusted analysis was performed as an

initial step where all variables were assessed individually for their association with Hb level/anaemia. Variables such as iron folic acid (IFA) intake, calcium supplementation, and dietary details were condensed into 2 categories for each variable in order to analyse the data in a manageable way for regression purposes. For example, dietary data were collected based on a weekly consumption pattern using the following four categories: Never, 1-2 times a week, 3-6 times a week and Everyday. The four categories were condensed in two (≤2 times a week, and ≥3 times week). The process provided sufficient participants in each category to conduct the analysis.

After the initial unadjusted analyses, a multivariable data analysis approach was developed. In the adjusted analysis individual risk factors based on their theoretical relationship with anaemia, and also with an understanding of the field settings were selected (Figure 17). During this model development, we also assessed multicollinearity of all factors using a covariate correlation matrix, and the variance inflation factor (VIF) [77]. Multicollinearity was only found between height, weight, and calculated body mass index; therefore, MUAC was the only anthropometric measurement included in the model (VIF <4). Additional information on each variable selection is outlined in detail in appendix 9.

Village level risk factors: Unadjusted analyses (logistic and linear regression) were conducted to investigate association of risk factors with anaemia, and Hb levels in pregnancy, respectively. An adjusted analysis was then performed to see the robustness of the associations observed (Figure 17). The village level covariates

were selected based on the understanding of the field area, and also their relevance to anaemia in pregnancy. Details on the selection of village factors are described in the appendix 10.

3. Risk factors associated with low birth weight

Post-delivery data were collected on various parameters including delivery term (preterm/full-term), delivery method (normal/assisted/caesarean section), neonatal outcomes (live birth/stillbirth/neonatal death), and pregnancy complications if observed. The descriptive analysis was conducted to summarise the study participant characteristics. Birth weight was the outcome of interest, and was used as a continuous measure for linear regression, while a binary categorical variable was used in the logistic regression (low birth weight: yes/no). Initially unadjusted analysis was performed, where all covariates were included. Adjusted analysis using pre-determined factors outlined below was then conducted to investigate the risk associated with low birth weight (Figure 17).

Figure 17: Multivariable data analysis approach for adjusted analysis

Pre delivery data- Individual risk factors for maternal anaemia assessment

Individual factors included in the regression analyses for anaemia risk assessment (10 covariates, outcome- maternal anaemia/Hb level): Maternal age, mid upper arm circumference (MUAC), currently consuming IFA supplementation, gravidity, 7-days diet recall, education completed, consanguineous marriage, current pregnancy registration at health facility, religion/category and annual income.

Village level risk factors for maternal anaemia assessment

Village level risk factors included in the regression analyses (10 covariates, outcome- maternal anaemia/Hb level): Water supplies, community toilets, government health centre, access to ASHA worker, availability of government nurse, services provided by the government nurse (Hb investigation, ANC), access to private doctor and travel time to nearby hospital.

Post-delivery data- Individual risk factors for low birth weight assessment

Individual factors included in the regression analyses for the birth weight assessment (10 covariates, outcome- low birth weight): Maternal age, mid upper arm circumference, currently consuming IFA supplementation, gravidity, 7-days diet recall, education completed, consanguineous marriage, current pregnancy registration at health facility, religion/category and annual income.

4. Comparison of non-invasive haemoglobin technology (NBM 200) with Sysmex XP-100 Hb measurements

Hb measurements provided by the automated analyser (Sysmex XP-100) were considered as the reference in this study, and those were compared with haemoglobin estimates generated from the NBM 200. Initially Spearman's coefficient correlation and the Bland-Altman analysis was calculated to measure level of agreement between two techniques [78].

Area Under Receiving Operating Characteristics (AUROC) was calculated to examine performance of the NBM 200. Additional analyses included sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) calculations to compare NBM 200 with the reference method.

- Sensitivity: The proportion of true positives correctly identified by NBM 200.
- Specificity: The proportion of true negatives correctly predicted by NBM 200.
- Positive predictive value (PPV): The proportion of NBM 200 anaemia positive participants for whom the anaemia diagnosis was confirmed by Sysmex XP-100.
- Negative predictive value (NPV): The proportion of NBM 200 anaemia negative participants who were non-anaemic as per the Sysmex XP-100.

Summary of statistical analysis is presented in figure 18.

Figure 18: Overview of statistical analysis

Individual anaemia risk factors during pregnancy

- Unadjusted analysis for all individual factors collected during the data collection.
- Adjusted analysis was conducted to see robustness of the association observed.
- Logistic and linear regression and results were presented with odds ratio/ β coefficient with 95% CI.

Village level risk factors related with maternal anaemia

- Unadjusted analysis of village factors relevant to pregnancy health in the study area.
- Adjusted analysis was conducted to see robustness of the association observed.
- Logistic and linear regression and results were presented in OR/ β coefficient with 95% CI.

Risk factors for low birth weight

- Unadjusted analysis was initially performed.
- Adjusted analysis was conducted to see robustness of the association observed.
- Logistic and linear regression and results were presented with odds ratio/ β coefficient with 95% CI.

NBM 200 validation

- Hb measurements from the NBM 200 were validated against gold standard measurement (Sysmex XP-100) using correlation, Bland-Altman analysis, sensitivity specificity and AUROC to evaluate NBM 200 in rural Indian setting.
- Sysmex XP-100 (venous blood measurement) was considered as the reference method.

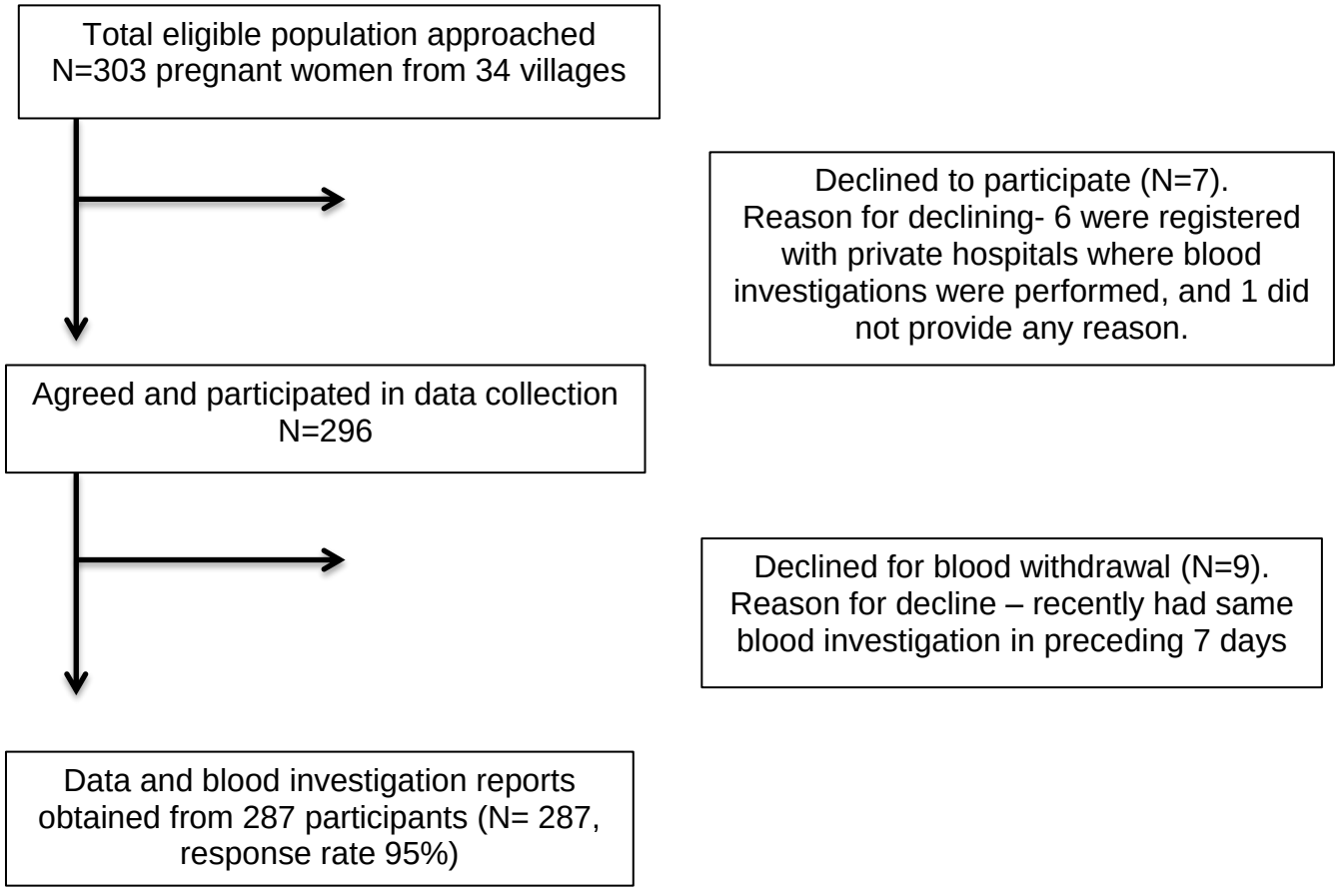
4.4 Results

The result section is divided in six segments: summary, individual risk factors associated with anaemia (pre-delivery data), village level risk factors associated with anaemia, birth weight analysis (post-delivery data), regression analysis summary, and NBM 200 validation.

4.4.1 Prevalence and severity of anaemia

During the recruitment period, we identified 303 eligible pregnancies from 34 villages, of whom 287 (study response rate: 95%) provided complete data (Figure 19). No pregnancies were identified from village named 'Arani'.

Figure 19: Flow chart of recruitment process (final sample size N= 287)



None of the participants reported any history of systemic disease/condition that could have influenced haemoglobin levels (for e.g. malaria, blood transfusion or hospital admissions) in the past 12 months at recruitment. None were diagnosed with hypertension during the baseline data collection. About 26% (of 287), were teenage pregnancies (maternal age ≤ 19 years, primiparas), and the anaemia prevalence did not vary by maternal age (age range 15 to 36 years) (Table 11). The majority of women (67%) had normal BMI (18.5 to 24.9 kg/m^2), while 28% were underweight (BMI < 18.5 kg/m^2).

Anaemia prevalence as defined by Hb < 11.0 g/dl was 77% (N=221, 95% CI: 72.1 to 81.9). About 3% had severe anaemia, 38% had moderate anaemia and 36% had mild anaemia. The mean haemoglobin was 10.5 g/dl (Standard Deviation (SD): 1.29, Range: 5.5 to 14, 95% CI: 9.9 to 10.2), and haemoglobin values followed a normal distribution (Figure 20). The mean of the mean corpuscular volume (MCV) was 80.5 fl/cell, (SD: 7.22, Range 53.4 to 93.8) (Figure 21) [116]. Other Red Blood Cell indices are detailed in the table 10. No abnormalities relating to white blood cells and platelets were detected. None of the participants were diagnosed with diabetes (Mean RBS: 70.78 mg/dl, SD 13.8).

Table 10: Red Blood Cell indices (N= 287, pre-delivery data)

Parameter	Mean	Standard Deviation	Range
Red Blood Cell Count	3.5 x 10^6/mm	0.36	2.7 to 4.9
Haemoglobin (Hb)	10.04 g/dl	1.29	5.5 to 14.0
Haematocrit	28.7%	2.85	20.2 to 38.5
Mean Corpuscular Haemoglobin (MCH)	28.2 pg/cell	3.63	14 to 35.1
Mean Corpuscular Volume (MCV)	80.5 fl/cell	7.22	53.4 to 93.8
Mean Corpuscular Haemoglobin Concentration (MCHC)	34.9 g/dl	1.82	26.2 to 38.8
RBC Distribution Width	42.5 fl	4.26	12.8 to 60.7
Reticulocytes %	14.3%	2.41	9.4 to 33.2

Table 10 footnotes: Measurement units used: RBC count was measured in million cells per microliter. Hb and MCHC were measured in grams per decilitre. Haemotocrit and Reticulocytes were measured in percentages (%). MCH was measured in pictograms per cell (pg/cell), while the MCV and RBC width were measured in femtolitres (fl) per cell. RBS was measured in milligram per decilitre.

Figure 20: Histogram of haemoglobin distribution among pregnant women (N=287, Hb in g/dl)

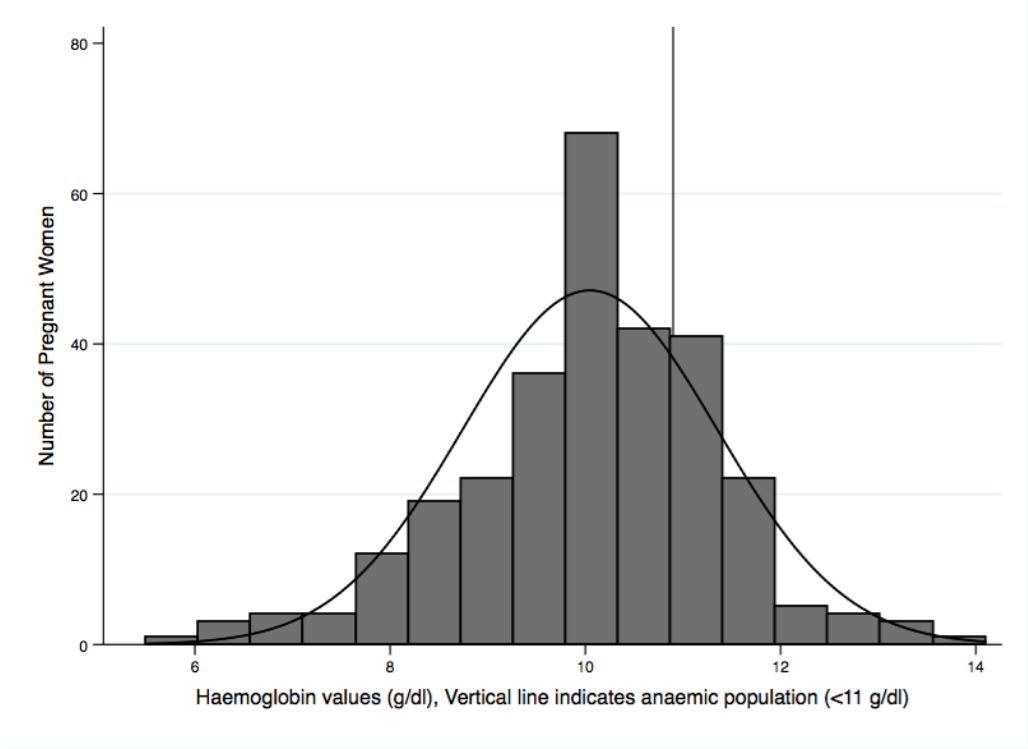
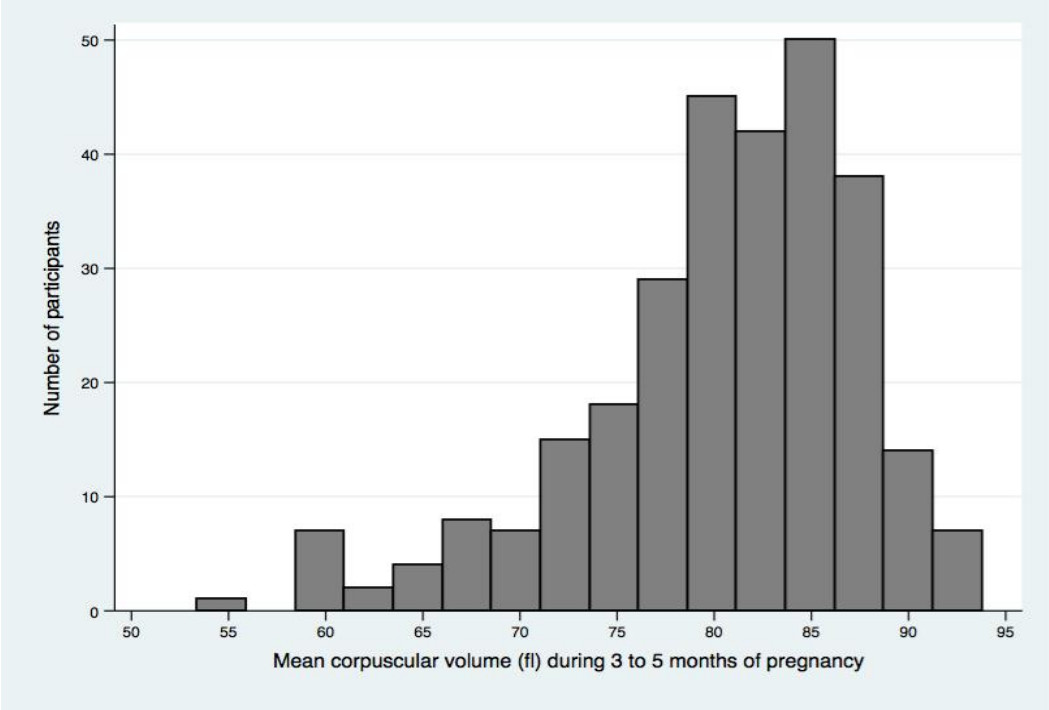


Figure 21: Histogram of mean corpuscular volume among pregnant women (N=287, MCV in fl/cell)



4.4.2 Individual risk factors associated with anaemia (pre-delivery data)

Unadjusted analysis

Linear regression reported decrease in Hb levels by 0.22 g/dl with monthly pregnancy progression (β coefficient -0.22 g/dl per month, 95% CI: 0.41 to -0.04, p=0.01) (Table 11). Medicines include IFA, Calcium and multivitamins were associated with low Hb levels. Household assets include television and vehicle were associated with high Hb levels. Logistic regression showed higher risks of anaemia among those who were in consanguineous marriage relationship (OR 2.21, 95% CI: 1.11 to 4.40, p=0.02).

Adjusted analysis

In the adjusted logistic regression consanguineous marriage was the only statistically significant risk factor associated with anaemia after adjustment for potential confounding factors (adjusted OR 2.41, 95% CI: 1.16 to 5.01, p = 0.02) (Table 12). None other studied factors reported significant association in the adjusted results.

Table 11: Characteristics of study population with unadjusted analyses (N= 287 participants)

Characteristics	Participants (%)	Non-anaemic participants (%)	Anaemic participants (%)	Linear regression with 95% CI [Hb levels in g/dl]	P value	Logistic regression with 95% CI	P value
Current pregnancy month							
3	77 (26.8)	24 (36.3)	53 (23.9)	-0.22 (-0.41 to -0.04)	0.01	1.28 (0.91 to 1.79)	0.14
4	92 (32.0)	17 (25.7)	75 (33.9)				
5	118 (41.1)	25 (37.8)	93 (42.0)				
Current age							
Up to 19 years	75 (26.1)	14 (21.2)	61 (27.6)	0.26 (-0.07 to 0.61)	0.12	0.70 (0.36 to 1.36)	0.30
20 and above	212 (73.8)	52 (78.7)	160 (72.3)				
Consanguineous marriage							
No	202 (70.3)	54 (81.8)	148 (66.9)	Ref		Ref	
Yes	85 (29.6)	12 (18.1)	73 (33.0)	-0.00 (-0.34 to 0.32)	0.96	2.21 (1.11 to 4.40)	0.02
Age at marriage							
Up to 19 years	236 (82.2)	52 (78.7)	184 (83.2)	0.06 (-0.33 to 0.46)	-0.75	0.74 (0.37 to 1.48)	-0.46
20 and above	51 (17.7)	14 (21.2)	35 (16.7)				
Education							
Primary school	170 (59.2)	38 (57.5)	132 (59.7)	Ref		Ref	
Secondary school	69 (24.0)	15 (22.7)	54 (24.4)	0.02 (-0.34 to 0.39)	0.90	1.03 (0.52 to 2.03)	0.91
Higher secondary school and above	48 (16.7)	13 (19.7)	35 (15.8)	0.02 (-0.39 to 0.44)	0.91	0.77 (0.32 to 1.61)	0.49
Last menstrual period known (LMP)							
No	10 (3.4)	4 (6.0)	6 (2.7)	Ref		Ref	
Yes	277 (96.5)	62 (93.9)	215 (97.2)	-0.27 (-1.10 to 0.55)	0.51	2.31 (0.63 to 8.45)	0.20
Body Mass Index (BMI) in kg/m^2							
Normal weight (18.5 to 24.9)	193 (67.2)	47 (71.2)	146 (66.0)	Ref		Ref	
Underweight (< 18.5)	82 (28.57)	15 (22.7)	67 (30.3)	-0.20 (-0.54 to 0.13)	0.22	1.43 (0.75 to 2.75)	0.27
Overweight (25.0 to 30.0)	12 (4.1)	4 (6.0)	8 (3.6)	0.04 (-0.72 to 0.81)	0.90	0.64 (0.18 to 2.23)	0.48

Mid upper arm circumference (MUAC)							
22 cm and above	262 (91.2)	60 (90.9)	202 (91.4)	Ref		Ref	
< 22 cm	25 (8.7)	6 (9.0)	19 (8.6)	-0.26 (-0.80 to 0.27)	0.33	0.94 (0.35 to 2.46)	0.90
Diagnosed with any diseases							
No	287 (100)	66 (100)	221 (100)	-	-	-	-
Yes	0	0 ()	0				
Currently taking iron folic acid supplements (IFA)							
No	74 (25.7)	19 (28.7)	55 (24.8)	Ref		Ref	
Yes	213 (74.2)	47 (71.2)	166 (75.1)	-0.17 (-0.52 to 0.17)	0.32	1.22 (0.66 to 2.25)	0.52
Currently taking calcium supplements							
No	156 (54.3)	42 (63.6)	114 (51.5)	Ref		Ref	
Yes	131 (45.6)	24 (36.3)	107 (48.4)	-0.32 (-0.62 to -0.02)	0.03	1.64 (0.93 to 2.89)	0.08
Currently taking multivitamins							
No	233 (81.1)	57 (86.3)	176 (79.6)	Ref		Ref	
Yes	54 (18.8)	9 (13.6)	45 (20.3)	-0.42 (-0.80 to -0.03)	0.03	1.61 (0.74 to 3.51)	0.22
Current gravidity							
1	128 (44.6)	26 (39.3)	102 (46.1)	-0.06 (-0.23 to 0.10)	0.45	1.02 (0.75 to 1.38)	0.89
2	103 (35.8)	30 (45.4)	73 (33.0)				
3 and more	56 (19.5)	10 (15.1)	46 (20.8)				
Total live births							
0	134 (46.6)	27 (40.9)	107 (48.4)	-0.03 (-0.23 to 0.17)	0.76	0.97 (0.67 to 1.39)	0.87
1	101 (35.1)	30 (45.4)	71 (32.1)				
2 and more	52 (18.12)	9 (13.6)	43 (19.4)				
Previous stillbirths							
No	282 (98.2)	64 (96.9)	218 (98.6)	Ref		Ref	
Yes	5 (1.7)	2 (3.0)	3 (1.3)	0.27 (-0.88 to 1.43)	0.63	0.44 (0.07 to 2.69)	0.37
Religion and category							
Hindu open category	137 (47.7)	31 (46.9)	106 (47.9)	Ref		Ref	
Hindu reserve category	127 (44.2)	28 (42.4)	99 (44.8)	-0.06 (-0.38 to 0.25)	0.68	1.03 (0.57 to 1.84)	0.91
Muslims	23 (8.0)	7 (10.6)	16 (7.2)	0.15 (-0.42 to 0.73)	0.60	0.66 (0.25 to 1.77)	0.41
Annual income (in INR)							

Less than 50,000	137 (47.7)	33 (50.0)	104 (47.0)	Ref		Ref	
Between 50,001 to 1,00,000	129 (44.9)	29 (43.9)	100 (45.2)	0.03 (-0.28 to 0.35)	0.82	1.09 (0.61 to 1.93)	0.75
More than 1,00,000	21 (7.3)	4 (6.0)	17 (7.6)	0.10 (-0.49 to 0.70)	0.73	1.34 (0.42 to 4.29)	0.61
House structure							
Permanent (pucca-concrete slab rooftop)	2 (0.7)	1 (1.5)	1 (0.4)	Ref		Ref	
Semi-Permanent (semi pucca- metal sheet rooftop)	265 (92.3)	62 (93.9)	203 (91.8)	-0.88 (-2.70 to 0.94)	0.34	3.27 (0.20 to 53.11)	0.40
Temporary (kutcha-wood/straw rooftop)	20 (6.9)	3 (4.5)	17 (7.6)	-1.28 (-3.18 to 0.62)	0.18	5.66 (0.27 to 117.44)	0.26
Farming land (in acres)							
No land	69 (24.0)	18 (27.2)	51 (23.0)	Ref		Ref	
Less than 5 acres	89 (31.0)	24 (36.3)	65 (29.4)	0.05 (-0.35 to 0.46)	0.79	0.95 (0.46 to 1.94)	0.90
More than 5 acres	129 (44.9)	24 (36.3)	105 (47.5)	-0.09 (-0.47 to 0.29)	0.64	1.54 (0.76 to 3.09)	0.22
Possess motor pump at farm							
No	150 (52.2)	39 (59.0)	111 (50.2)	Ref		Ref	
Yes	137 (47.7)	27 (40.9)	110 (49.7)	-0.12 (-0.42 to 0.17)	0.41	1.43 (0.82 to 2.49)	0.20
Possess motorbike							
No	181 (63.0)	40 (60.6)	141 (63.8)	Ref		Ref	
Yes	106 (36.9)	26 (39.3)	80 (36.2)	0.09 (-0.22 to 0.40)	0.56	0.87 (0.49 to 1.53)	0.63
Possess car/vehicle							
No	250 (87.1)	52 (78.7)	198 (89.5)	Ref		Ref	
Yes	37 (12.8)	14 (21.2)	23 (10.4)	0.48 (0.03 to 0.93)	0.03	0.43 (0.20 to 0.89)	0.02
Possess television							
No	90 (31.3)	18 (27.2)	72 (32.5)	Ref		Ref	
Yes	197 (68.6)	48 (72.7)	149 (67.4)	0.32 (0.00 to 0.65)	0.04	0.77 (0.42 to 1.42)	0.41
Possess mobile							
No	10 (3.4)	3 (4.5)	7 (3.1)	Ref		Ref	
Yes	277 (96.5)	63 (95.4)	214 (96.8)	-0.17 (-1.00 to 0.64)	0.67	1.45 (0.36 to 5.79)	0.59
Possess livestock							
No	78 (27.1)	22 (33.3)	56 (25.3)	Ref		Ref	

Yes	209 (72.9)	44 (66.6)	165 (74.6)	0.06 (-0.27 to 0.41)	0.68	1.47 (0.81 to 2.67)	0.20
Registered under below poverty families (BPL)							
No	179 (62.3)	44 (66.6)	135 (61.0)	Ref		Ref	
Yes	108 (37.6)	22 (33.3)	86 (38.9)	-0.19 (-0.50 to 0.11)	0.22	1.27 (0.71 to 2.27)	0.41
Sold any assets in past 12 months (assets such as land, house, gold, valuables)							
No	238 (82.9)	54 (81.8)	184 (83.2)	Ref		Ref	
Yes	49 (17.0)	12 (18.1)	37 (16.7)	0.03 (-0.36 to 0.44)	0.84	0.90 (0.44 to 1.85)	0.78
Dietary preferences							
Vegetarian	118 (41.1)	30 (45.4)	88 (39.8)	Ref		Ref	
Eggetarian	30 (10.4)	1 (1.5)	29 (13.1)	-0.37(-0.89 to 0.15)	0.16	9.88 (1.29 to 75.73)	0.02
Non-vegetarian	139 (48.4)	35 (53.0)	104 (47.0)	-0.00 (-0.33 to 0.31)	0.95	1.01 (0.57 to 1.78)	0.96
Diet recall (last seven days history)							
Pure Milk (not in the form of tea/coffee)							
≤ 2 times a week	159 (55.4)	37 (56.0)	122 (55.2)	Ref		Ref	
≥ 3 times a week	128 (44.6)	29 (43.9)	99 (44.8)	0.10 (-0.19 to 0.41)	0.49	1.03 (0.59 to 1.80)	0.90
Green leafy vegetables							
≤ 2 times a week	165 (57.4)	35 (53.0)	130 (58.8)	Ref		Ref	
≥ 3 times a week	122 (42.5)	31 (46.9)	91 (41.1)	-0.01 (-0.31 to 0.29)	0.93	0.79 (0.45 to 1.37)	0.40
Bean sprouts							
≤ 2 times a week	278 (96.8)	64 (96.9)	214 (96.8)	Ref		Ref	
≥ 3 times a week	9 (3.1)	2 (3.0)	7 (3.1)	0.28 (-0.58 to 1.15)	0.52	1.04 (0.21 to 5.16)	0.95
Pulses-lentils							
≤ 2 times a week	74 (25.7)	15 (22.7)	59 (26.7)	Ref		Ref	
≥ 3 times a week	213 (74.2)	51 (77.2)	162 (73.3)	0.06 (-0.28 to 0.40)	0.72	0.80 (0.42 to 1.54)	0.51
Fruits/juices							
≤ 2 times a week	154 (53.6)	36 (54.5)	118 (53.3)	Ref		Ref	
≥ 3 times a week	133 (46.3)	30 (45.4)	103 (46.6)	-0.17 (-0.47 to 0.13)	0.26	1.04 (0.60 to 1.81)	0.86
Rice							
≤ 2 times a week	20 (6.9)	4 (6.0)	16 (7.2)	Ref		Ref	
≥ 3 times a week	267 (93.0)	62 (93.9)	205 (92.7)	0.30 (-0.29 to 0.89)	0.31	0.82 (0.26 to 2.56)	0.74
Eggs							
≤ 2 times a week	271 (94.4)	62 (93.9)	209 (94.5)	Ref		Ref	
≥ 3 times a week	16 (5.5)	4 (6.0)	12 (5.4)	0.16 (-0.50 to 0.82)	0.63	0.88 (0.27 to 2.85)	0.84

Chicken							
≤ 2 times a week	279 (97.2)	65 (98.4)	214 (96.8)	Ref		Ref	
≥ 3 times a week	8 (2.7)	1 (1.5)	7 (3.1)	-0.20 (-1.12 to 0.72)	0.66	2.12 (0.25 to 17.60)	0.48
Goat meat							
≤ 2 times a week	284 (98.9)	65 (98.4)	219 (99.1)	Ref		Ref	
≥ 3 times a week	3 (1.0)	1 (1.5)	2 (0.9)	0.92 (-0.56 to 2.42)	0.22	0.59 (0.05 to 6.65)	0.67
Cooking fuel							
Smoke free (LPG, electric range, kerosene)	52 (18.1)	13 (19.7)	39 (17.6)	Ref		Ref	
Causes smoke (wood/straw/animal dung)	235 (81.8)	53 (80.3)	182 (82.3)	-0.18 (-0.57 to 0.20)	0.35	1.14 (0.56 to 2.30)	0.70
Heard about anaemia and Haemoglobin							
No	209 (72.8)	44 (66.6)	165 (74.6)	Ref		Ref	
Yes	78 (27.1)	22 (33.3)	56 (25.3)	0.10 (-0.23 to 0.44)	0.54	0.67(0.37 to 1.23)	0.20
Checked haemoglobin during current pregnancy							
No	124 (43.2)	29 (43.9)	95 (42.9)	Ref		Ref	
Yes	163 (56.7)	37 (56.0)	126 (57.0)	-0.11 (-0.42 to 0.18)	0.45	1.03 (0.59 to 1.80)	0.89
Know recent haemoglobin levels							
No	223 (77.7)	52 (78.7)	171 (77.3)	Ref		Ref	
Yes	64 (22.3)	14 (21.2)	50 (22.6)	-0.27(-0.63 to 0.09)	0.14	1.08 (0.55 to 2.12)	0.80
Pregnancy registration							
Registered with government or private hospital	269 (93.7)	62 (93.9)	207 (93.6)	Ref		Ref	
Not registered with any hospital	18 (6.2)	4 (6.0)	14 (6.3)	-0.14 (-0.77 to 0.48)	0.64	1.04 (0.33 to 3.30)	0.93

Table 11 footnotes:

- p<0.05, ** p<0.001
- Annual incomes expressed in GBP: Great Britain Pound (1 GBP= 100 Indian Rupees)
- Religion and categories are important component of Indian societal structure. Category represents ethnic background, which are mainly divided into open and reserved classes.

- For linear regression Hb values were used as a continuous outcome, and binary category variable (anaemia status yes/no) is used in logistic regression. Ref indicates reference category
- Maternal anaemia was defined as a haemoglobin level less than 11.0 g/dl.

Table 12: Pre-delivery data (multivariable model): risk factors associated with Hb levels (linear regression) and anaemia (logistic regression) in pregnant women (N= 287)

Characteristics	Unadjusted linear regression β (95% CI) [Hb levels in g/dl]	Adjusted linear regression β (95% CI) [Hb levels in g/dl]	Unadjusted logistic regression OR (95% CI)	Adjusted logistic regression OR (95% CI)
Maternal age	0.01 (-0.04 to 0.06)	0.01 (-0.04 to 0.08)	0.94 (0.86 to 1.03)	0.92 (0.82 to 1.04)
Mid-upper arm circumference of dominant hand (MUAC)				
< 22	Ref	Ref	Ref	Ref
≥ 22 cm and above	0.26 (-0.27 to 0.80)	0.21 (-0.35 to 0.77)	1.06 (0.40 to 2.78)	1.13 (0.41 to 3.12)
Currently consuming Iron Folic Acid supplements				
No	Ref	Ref	Ref	Ref
Yes	-0.17 (-0.52 to 0.17)	-0.28 (-0.68 to 0.11)	1.22 (0.66 to 2.25)	1.44 (0.70 to 2.93)
Gravidity	-0.04 (-0.21 to 0.12)	-0.08 (-0.30 to 0.13)	0.98 (0.73 to 1.34)	1.03 (0.69 to 1.53)
Diet recall (recent 7-days history)				
Pure milk (not in the form of tea/coffee)				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.10 (-0.19 to 0.41)	0.12 (-0.21 to 0.46)	1.03 (0.59 to 1.80)	1.02 (0.54 to 1.89)
Green leafy vegetables				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	-0.01 (-0.31 to 0.29)	-0.01 (-0.34 to 0.31)	0.79 (0.45 to 1.37)	0.77 (0.42 to 1.41)
Bean sprouts				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.28 (-0.58 to 1.15)	0.18 (-0.73 to 1.10)	1.04 (0.21 to 5.16)	1.05 (0.28 to 8.04)
Pulses-lentils				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.06 (-0.28 to 0.40)	0.00 (-0.36 to 0.38)	0.80 (0.42 to 1.54)	0.87 (0.43 to 1.75)
Fruits/Fruit juices				

≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	-0.17 (-0.47 to 0.13)	-0.18 (-0.50 to 0.13)	1.04 (0.60 to 1.81)	1.09 (0.61 to 1.96)
Rice				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.30 (-0.29 to 0.89)	0.26 (-0.36 to 0.90)	0.82 (0.26 to 2.56)	1.14 (0.34 to 3.83)
Eggs				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.16 (-0.50 to 0.82)	0.18 (-0.52 to 0.89)	0.88 (0.27 to 2.85)	0.77 (0.22 to 2.67)
Chicken				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	-0.20 (-1.12 to 0.72)	-0.15 (-1.12 to 0.81)	2.12 (0.25 to 17.60)	2.09 (0.23 to 18.44)
Goat meat				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.92 (-0.56 to 2.42)	0.95 (-0.61 to 2.52)	0.59 (0.05 to 6.65)	0.57 (0.04 to 7.83)
Education completed				
Primary school	Ref	Ref	Ref	Ref
Secondary school	0.02 (-0.34 to 0.39)	0.05 (-0.34 to 0.46)	1.03 (0.52 to 2.03)	0.88 (0.41 to 1.86)
≥ Higher secondary school	0.02 (-0.39 to 0.44)	0.00 (-0.46 to 0.47)	0.77 (0.37 to 1.61)	0.78 (0.34 to 1.77)
Consanguineous marriage				
No	Ref	Ref	Ref	Ref
Yes	-0.00 (-0.34 to 0.32)	0.00 (-0.35 to 0.36)	2.21 (1.11 to 4.40)*	2.41 (1.16 to 5.01)*
Current pregnancy registration at health facility				
No	Ref	Ref	Ref	Ref
Yes	0.14 (-0.48 to 0.77)	0.31 (-0.39 to 1.02)	0.95 (0.30 to 3.00)	0.75 (0.20 to 2.70)
Religion and category				
Hindu open category	Ref	Ref	Ref	Ref

Hindu reserved category	-0.06 (-0.38 to 0.25)	-0.04 (-0.41 to 0.31)	1.03 (0.57 to 1.84)	0.99 (0.51 to 1.92)
Muslim	0.15 (-0.42 to 0.73)	0.06 (-0.57 to 0.71)	0.66 (0.25 to 1.77)	0.67 (0.22 to 2.01)
Annual income				
≤ 500 GBP	Ref	Ref	Ref	Ref
Between 501 and 1000 GBP	0.03 (-0.28 to 0.35)	0.01 (-0.33 to 0.35)	1.09 (0.61 to 1.93)	1.10 (0.59 to 2.05)
≥ 1001 GBP	0.10 (-0.49 to 0.70)	0.02 (-0.64 to 0.69)	1.34 (0.42 to 4.29)	1.47 (0.42 to 5.18)

Table 12 footnotes:

- *p<0.05, OR=odds ratio, β=linear regression correlation coefficient, CI= confidence interval.
- Annual income is expressed in Great Britain Pound (GBP). 1 GBP was calculated as being equivalent to 100 INR.
- Maternal age and gravidity were used as continuous measures following linearity assessments.
- Ref indicates reference category.

4.4.3 Village level risk factors associated with anaemia

Of 35 study villages, no pregnancies were identified from a village named ‘Arani’ as outlined earlier, thus the rest 34 villages were considered in the risk analysis.

All villages (N=34) were served by government nurses, but the majority of them (N=29) had access for one-day a month, when nurses used to visit for ANC check-ups (vaccinations and IFA supplementation). In the other 5 villages, pregnant women had daily access to healthcare staff as nurses were stationed in the same village. Normal obstetric delivery facilities were available only in one village. Of 34, 24 village authorities were providing piped water supplies to households, and in the 11 villages community toilets were in use. All villages had limited transport services to the nearest secondary care hospital, and were mostly serviced by private vehicle operators.

Mean haemoglobin was more than 11.0 g/dl (non-anaemic) for two villages. The remaining 32 villages had mean Hb between 8.5 g/dl to 10.5 g/dl (mild and moderate anaemia). No village was observed with mean below 8.0 g/dl (Figure 22).

Unadjusted analysis

Daily access to a government nurse was the only factor that showed significant association with anaemia, when assessed in the unadjusted regression. Access to nurse reduced anaemia risk by 52% (OR 0.48, 95% CI: 0.25 to 0.93, p=0.03) (Table 13).

Adjusted analysis

None of the village level risk factor reported significant association with maternal anaemia/ haemoglobin levels on conducting full adjustments (Table 14).

Figure 22: Haemoglobin distribution in study villages (N=34 villages)

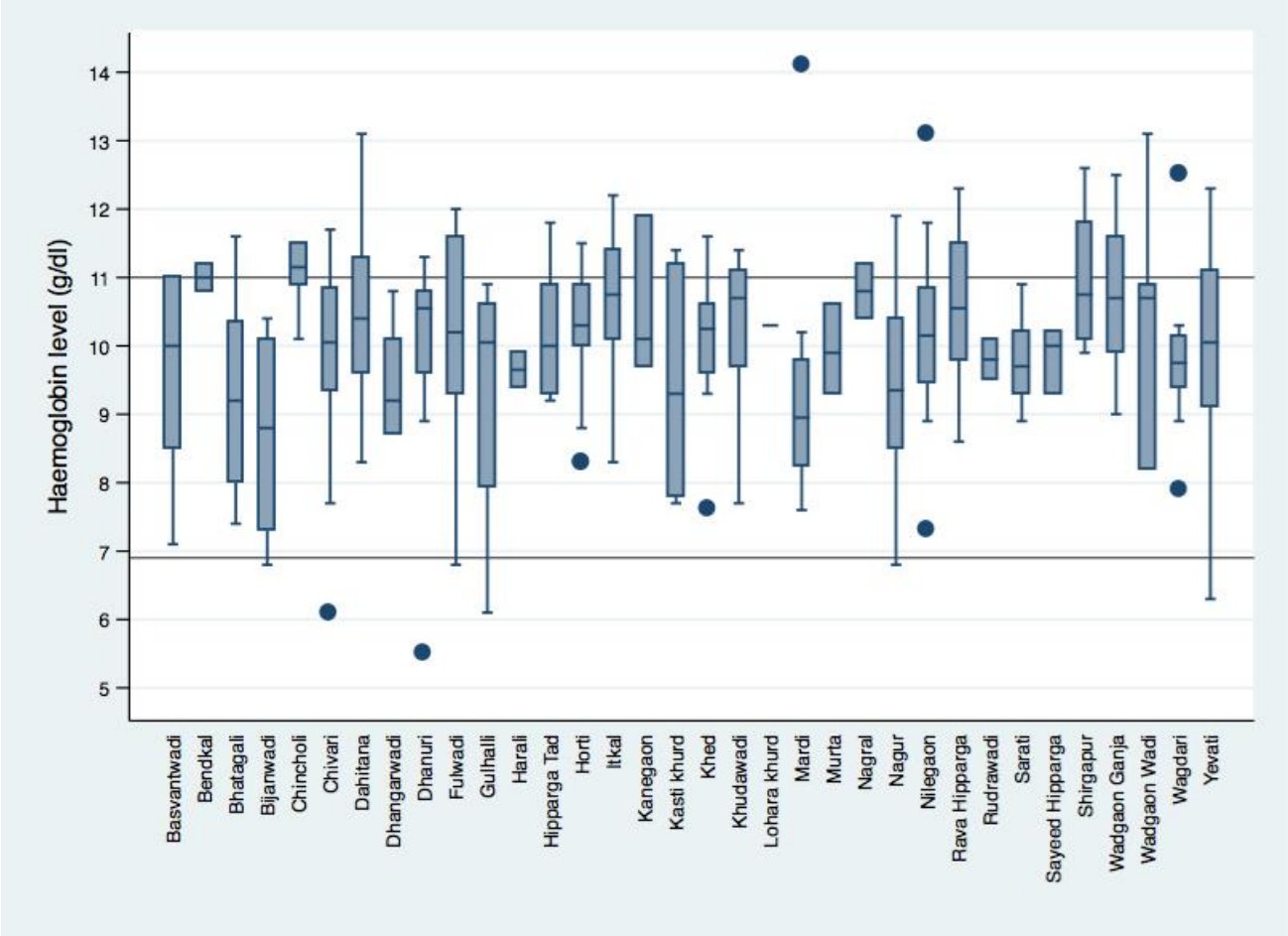


Figure 22 footnotes:

- Villages plotted on X axis and haemoglobin values on Y-axis. Y-axis horizontal reference lines: At 11.0 g/dl, area above indicates non-anaemic population, and at 6.9 g/dl and area below indicates severe anaemic population. Area between these two reference lines represents the population with mild and moderate anaemia. Dots are outliers.

Table 13: Characteristics of villages with unadjusted analyses (N= 34 villages)

Village Characteristics	Village (%)	Non-anaemic participants (%)	Anaemic participants (%)	Linear regression, β coefficient (95% confidence interval) [Hb levels in g/dl]		Logistic regression, odds ratio (95% confidence interval)	
Piped water supplied to houses							
No	10 (29.4)	18 (27.2)	50 (22.6)	Ref	P value	Ref	P value
Yes	24 (70.5)	48 (72.7)	171 (77.3)	-0.22 (-0.58 to 0.13)	0.21	1.28 (0.68 to 2.40)	0.43
Daily water supply (at least once a day)							
No	7 (20.5)	10 (15.1)	46 (20.8)	Ref		Ref	
Yes	27 (79.4)	56 (84.8)	175 (79.1)	0.24 (-0.14 to 0.62)	0.21	0.67 (0.32 to 1.43)	0.31
Operational community toilets							
No	23 (67.6)	23 (34.8)	99 (44.8)	Ref	-	Ref	-
Yes	11 (32.3)	43 (65.1)	122 (55.2)	0.10 (-0.20 to 0.40)	0.51	0.65 (0.37 to 1.16)	0.15
Government health centre availability							
No	24 (70.5)	36 (54.5)	141 (63.8)	Ref	-	Ref	-
Yes	10 (29.4)	30 (45.4)	80 (36.2)	0.22 (-0.08 to 0.54)	0.14	0.68 (0.39 to 1.18)	0.17
Accredited social health attendant (ASHA) appointed							
No	1 (2.9)	2 (3.0)	4 (1.8)	Ref	-	Ref	-
Yes	33 (97.0)	64 (96.9)	217 (98.1)	-0.95 (-2.01 to 0.10)	0.07	1.69 (0.30 to 9.46)	0.54
Daily access to government nurse							
No	29 (85.2)	48 (72.7)	187 (84.6)	Ref		Ref	
Yes	5 (14.7)	18 (27.2)	34 (15.3)	0.24 (-0.14 to 0.63)	0.22	0.48 (0.25 to 0.93)*	0.03
Government nurse provides antenatal care services							
No	1 (2.9)	2 (3.0)	4 (1.8)	Ref	-	Ref	-
Yes	33 (97.0)	64 (96.9)	217 (98.1)	0.45 (-0.60 to 1.51)	0.39	1.69 (0.30 to 9.46)	0.54
Government nurse conducts haemoglobin investigation							
No	8 (23.5)	14 (21.2)	54 (24.4)	Ref	-	Ref	-
Yes	26 (76.4)	52 (78.7)	167 (75.5)	-0.13 (-0.49 to 0.22)	0.45	0.83 (0.42 to 1.61)	0.58
Private doctor availability							

No	31 (91.1)	52 (78.7)	184 (83.2)	Ref	-	Ref	-
Yes	3 (8.8)	14 (21.2)	37 (16.7)	0.34 (-0.04 to 0.74)	0.08	0.74 (0.37 to 1.48)	0.40
Travel time to nearest hospital	Continuous variable	-	-	-0.00 (-0.00 to 0.01)	0.82	1.00 (0.98 to 1.02)	0.62

Table 13 footnotes:

- For linear regression Hb values were used as a continuous outcome, and binary category variable (anaemia status yes/no) is used in logistic regression. Ref indicates reference category.
- Maternal anaemia was defined as a haemoglobin level less than 11.0 g/dl.

Table 14: Adjusted regression results (multivariable model): village level risk factors associated with anaemia in pregnant women (N= 287 women from 34 villages)

Characteristics	Unadjusted linear regression β (95% CI) [Hb levels in g/dl]	Adjusted linear regression β (95% CI) [Hb levels in g/dl]	Unadjusted logistic regression OR (95% CI)	Adjusted logistic regression OR (95% CI)
Piped water supplied to houses				
No	Ref	Ref	Ref	Ref
Yes	-0.22 (-0.58 to 0.13)	-0.37 (-0.81 to 0.07)	1.28 (0.68 to 2.40)	1.70 (0.72 to 3.98)
Daily water supply (at least once a day)				
No	Ref	Ref	Ref	Ref
Yes	0.24 (-0.14 to 0.62)	-0.01 (-0.44 to 0.41)	0.67 (0.32 to 1.43	0.92 (0.38 to 2.22)
Operational community toilets				
No	Ref	Ref	Ref	Ref
Yes	0.10 (-0.20 to 0.40)	0.18 (-0.19 to 0.55)	0.65 (0.37 to 1.16)	0.53 (0.25 to 1.11)
Government health centre availability (sub-centre/primary health centre)				
No	Ref	Ref	Ref	Ref
Yes	0.22 (-0.08 to 0.54)	0.25 (-0.28 to 0.79)	0.68 (0.39 to 1.18)	0.91 (0.32 to 2.55)
Accredited social health attendant (ASHA) appointed				
No	Ref	Ref	Ref	Ref
Yes	-0.95 (-2.01 to 0.10)	-0.98 (-2.10 to 0.14)	1.69 (0.30 to 9.46)	2.01 (0.31 to 13.02)
Daily access to government nurse				
No	Ref	Ref	Ref	Ref
Yes	0.24 (-0.14 to 0.63)	0.10 (-0.40 to 0.61)	0.48 (0.25 to 0.93)	0.48 (0.19 to 1.19)
Government nurse provides ANC services				
No	Ref	Ref	Ref	Ref
Yes	0.45 (-0.60 to 1.51)	0.42 (-0.73 to 1.59)	1.69 (0.30 to 9.46)	1.95 (0.26 to 14.36)
Government nurse conducts haemoglobin investigation				
No	Ref	Ref	Ref	Ref
Yes	-0.13 (-0.49	-0.25 (-0.70	0.83 (0.42 to	0.99 (0.39

	to 0.22)	to 0.20)	1.61)	to 2.48)
Private doctor availability				
No	Ref	Ref	Ref	Ref
Yes	0.34 (-0.04 to 0.74)	0.26 (-0.22 to 0.75)	0.74 (0.37 to 1.48)	0.93 (0.40 to 2.20)
Travel time to the nearest primary care hospital (continuous variable)	-0.00 (-0.00 to 0.01)	0.00 (-0.01 to 0.02)	1.00 (0.98 to 1.02)	0.99 (0.95 to 1.04)

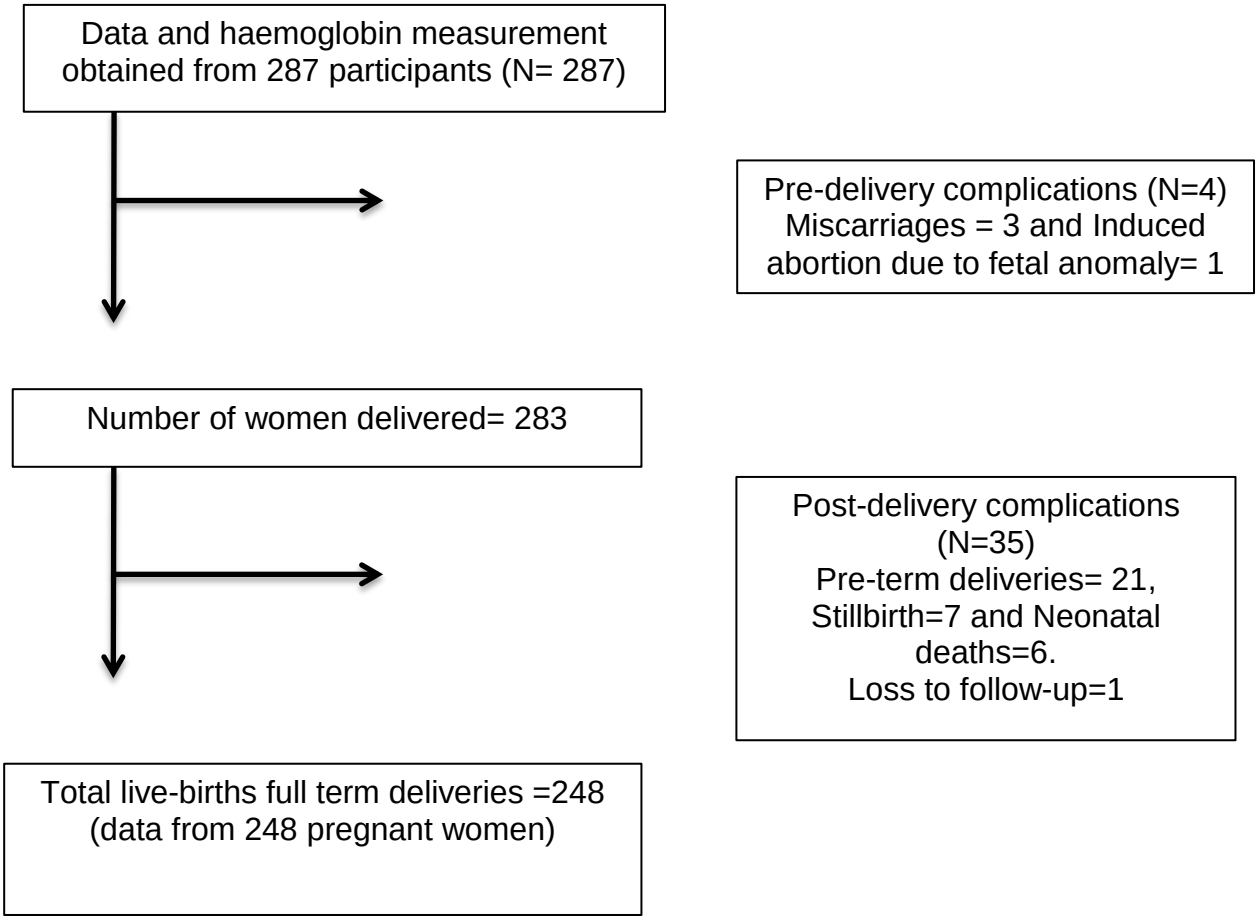
Table 14 footnotes:

- *p<0.05 **p<0.001 (if found to be significant).
- Maternal anaemia was defined as a haemoglobin level less than 11.0 g/dl.
- Ref indicates reference category.

4.4.4 Birth weight analysis (post-delivery data)

Of 287 women recruited at baseline, 86% (248) had full term singleton live births without any maternal or neonatal complications (Figure 23). Of 35 participants identified with complications, 11 women were in consanguineous relationships (where 7 preterm delivery, 2 stillbirths and 2 neonatal deaths were recorded). No maternal deaths were recorded in the study. None of the participants were diagnosed with systemic conditions such as hypertension or diabetes during pregnancy.

Figure 23: Participants considered in the post-delivery birth weight analysis (final sample size N=248)



Of 248 full-term singleton live births, 211 (85%) were vaginal deliveries, and 37 (15%) were caesarean sections. In total, 236 participants (95%) delivered in a healthcare facility, and the remaining 12 (5%) were home deliveries (Table 15). There were 18 (7%) low birth weight (LBW) babies (95% CI: 4.0 to 10.5), and the mean birth weight was 2.89 Kg (SD: 0.42 Kg, 95% CI: 2.8 to 2.9), and 49% (123/248) were female. No significant difference was seen in the odds of LBW or mean birth weight by babies' sex.

Unadjusted analysis

Maternal anaemia and consanguineous marriage were the only two factors showing an association with low birth weight. There was a statistically significant higher likelihood of LBW among women who had a consanguineous marriage (unadjusted OR 3.13, 95% CI: 1.25 to 8.77, p=0.01) compared to those who did not. The presence of maternal anaemia in the third to fifth months of pregnancy was associated with lower risk of LBW (unadjusted OR 0.34, 95% CI: 0.13 to 0.92, p= 0.03) (Table 15).

Adjusted analysis

Consanguineous marriage showed significant association with low birth weight in the adjusted analysis (adjusted OR 4.10, 95% CI: 1.24 to 13.41, p=0.02) (Table 16). The analysis was performed using multivariable modelling strategy using 10 maternal covariates outlined previously (Figure 17).

Maternal anaemia was not included in this analysis considering it could be on the causal pathway modifying the risk observed. The association of anaemia with LBW remained

significant when adjusted independently only for consanguineous marriage (OR 0.25, 95% CI: 0.09 to 0.73, p=0.01). On conducting a linear regression of two continuous variables of interest, birth weight (outcome) and maternal haemoglobin (exposure), a borderline significant association was observed (β -0.36 Kg, 95% CI: -0.74 to 0.02, p=0.06).

Table 15: Post-delivery data: individual risk factors associated with low birth weight (unadjusted analysis) (N= 248 pregnant women)

Characteristics	Participants f (%)	Normal birth weight babies (%)	Low birth weight babies (%)	Linear regression with 95% CI [birth weight in Kg]	P value	Logistic regression with 95% CI	P value
Pregnancy month at the time of recruitment							
3	69 (27.8)	62 (26.9)	7 (38.8)	0.05 (-0.00 to 0.12)	0.07	0.90 (0.50 to 1.62)	0.73
4	81 (32.6)	78 (33.9)	3 (16.6)				
5	98 (39.5)	90 (39.1)	8 (44.4)				
Maternal age							
Up to 19 years	63 (25.4)	57 (24.7)	6 (33.3)	0.00 (-0.01 to 0.01)	0.90	0.98 (0.83 to 1.16)	0.87
20 and above	185 (74.5)	173 (75.2)	12 (66.6)				
Consanguineous marriage							
No	175 (70.5)	167 (72.6)	8 (44.4)	Ref		Ref	
Yes	73 (29.4)	63 (27.3)	10 (55.5)	-0.11 (-0.23 to -0.00)*	0.04*	3.13 (1.25 to 8.77)	0.01*
Age at marriage							
Up to 19 years	202 (81.4)	185 (80.4)	17 (94.4)	0.00 (-0.01 to 0.03)	0.52	0.79 (0.60 to 1.03)	0.09
20 and above	46 (18.5)	45 (19.5)	1 (5.5)				
Education							
Primary school	138 (55.6)	125 (54.3)	13 (72.2)	Ref		Ref	
Secondary school	66 (26.6)	61 (26.5)	5 (27.7)	0.00 (-0.12 to 0.12)	0.96	0.78 (0.26 to 2.31)	0.66
Higher secondary school and above	44 (17.7)	44 (19.1)	0	0.07 (-0.07 to 0.21)	0.32	Omitted	
Last menstrual period known (LMP)							
No	7 (2.8)	6 (2.6)	1 (5.5)	Ref		Ref	
Yes	241 (97.1)	224 (97.3)	17 (94.4)	-0.02 (-0.34 to 0.30)	0.90	0.45 (0.05 to 4.00)	0.47
Body Mass Index (BMI) at the time of recruitment in kg/m^2							
Normal weight (18.5 to 24.9)	72 (29.0)	66 (28.7)	6 (33.3)	Ref		Ref	

Underweight (< 18.5)	165 (66.5)	154 (66.9)	11 (61.1)	0.11 (-0.00 to 0.23)	0.05	0.78 (0.27 to 2.21)	0.64
Overweight (25.0 to 30.0)	11 (4.4)	10 (4.3)	1 (5.5)	0.28 (-0.01 to 0.55)	0.04*	1.10 (0.11 to 10.11)	0.93
Mid upper arm circumference (MUAC) at the time of recruitment							
22 cm and above	226 (91.1)	20 (8.7)	2 (11.1)	Ref		Ref	
< 22 cm	22 (8.8)	210 (91.3)	16 (88.8)	0.11 (-0.06 to 0.30)	0.21	0.76 (0.16 to 3.55)	0.72
Diagnosed diseases							
No	248 (100)	230 (100)	18 (100)	Omitted	-	Omitted	-
Yes	0			-	-	-	-
Iron folic acid supplements (IFA) at the time of recruitment							
No	58 (23.3)	51 (2.1)	7 (38.8)	Ref		Ref	
Yes	190 (76.6)	179 (77.8)	11 (61.1)	0.05 (-0.06 to 0.18)	0.37	0.44 (0.16 to 1.21)	0.11
Calcium supplements at the time of recruitment							
No	133 (53.6)	124 (53.9)	9 (50)	Ref		Ref	
Yes	115 (46.3)	106 (46.0)	9 (50)	0.00 (-0.09 to 0.11)	0.86	1.16 (0.44 to 3.05)	0.74
Multivitamins at the time of recruitment							
No	197 (79.4)	183 (79.5)	14 (77.7)	Ref		Ref	
Yes	51 (20.5)	47 (20.4)	4 (22.2)	-0.00 (-0.13 to 0.12)	0.92	1.11 (0.34 to 3.53)	0.85
Current gravidity							
1	109 (43.90	102 (44.3)	7 (38.8)	0.02 (-0.03 to 0.07)	0.46	0.79 (0.44 to 1.41)	0.43
2	89 (35.8)	79 (34.3)	10 (55.5)				
3 and more	50 (20.1)	49 (21.3)	1 (5.5)				
Previous live births							
0	115 (46.3)	108 (46.9)	7 (38.8)	0.02 (-0.03 to 0.08)	0.41	0.84 (0.47 to 1.49)	0.56
1	87 (35.0)	77 (33.4)	10 (55.5)				
2 and more	46 (18.5)	45 (13.9)	1 (5.5)				
Previous stillbirths							
No	243 (97.9)	225 (97.8)	18 (100)	Ref		Omitted	-
Yes	5 (2.0)	5 (2.1)	0	-0.04 (-0.42 to 0.33)	0.81	-	-
Religion and category							
Hindu open category	128 (51.6)	120 (52.1)	8 (44.4)	Ref		Ref	
Hindu reserve category	105 (42.3)	96 (41.7)	9 (50)	0.01 (-0.09 to 0.12)	0.74	1.40 (0.52 to 3.78)	0.49

Muslims	15 (6.0)	14 (6.0)	1 (5.5)	0.10 (-0.12 to 0.33)	0.34	1.07 (0.12 to 9.20)	0.95
Annual income (in INR)							
Less than 50,000	113 (45.5)	105 (45.6)	8 (44.4)	Ref		Ref	
Between 50,001 to 1,00,000	117 (47.1)	107 (46.5)	10 (55.5)	-0.05 (-0.16 to 0.05)	0.36	1.22 (0.45 to 3.22)	0.64
More than 1,00,000	18 (7.2)	18 (7.8)	0	0.06 (-0.15 to 0.27)	0.57	Omitted	-
House structure							
Permanent (pucca- concrete slab rooftop)	2 (0.8)	2 (0.8)	0	Ref		Omitted	-
Semi-Permanent (semi pucca- metal sheet rooftop)	228 (91.9)	212 (92.1)	16 (88.8)	-0.10 (-0.70 to 0.49)	0.73	-	-
Temporary (kutcha- wood/straw rooftop)	18 (7.2)	16 (6.9)	2 (11.1)	-0.15 (-0.78 to 0.46)	0.61	-	-
Farming land (in acres)							
No land	52 (20.9)	49 (21.3)	3 (16.6)	Ref		Ref	
Less than 5 acres	82 (33.0)	72 (31.3)	10 (55.5)	-0.04 (-0.19 to 0.09)	0.51	2.26 (0.59 to 8.66)	0.23
More than 5 acres	114 (45.9)	109 (47.3)	5 (27.7)	-0.04 (-0.18 to 0.09)	0.55	0.74 (0.17 to 3.26)	0.70
Possess motor pump at farm							
No	124 (50)	116 (50.4)	8 (44.4)	Ref		Ref	
Yes	124 (50)	114 (49.5)	10 (55.5)	-0.00 (-0.11 to 0.09)	0.88	1.27 (0.48 to 3.33)	0.62
Possess motorbike							
No	153 (61.6)	142 (61.7)	11 (61.1)	Ref		Ref	
Yes	95 (38.3)	88 (38.2)	7 (38.8)	0.09 (-0.01 to 0.20)	0.08	1.02 (0.38 to 2.74)	0.95
Possess car/vehicle							
No	213 (85.8)	198 (86.0)	15 (83.3)	Ref		Ref	
Yes	35 (14.1)	32 (13.9)	3 (16.6)	0.04 (-0.10 to 0.19)	0.56	1.23 (0.33 to 4.51)	0.74
Possess television							
No	71 (28.6)	67 (29.1)	4 (22.2)	Ref		Ref	
Yes	177 (71.3)	163 (70.8)	14 (77.7)	-0.02 (-0.14 to 0.09)	0.65	1.43 (0.45 to 4.53)	0.53
Possess mobile							
No	8 (3.2)	6 (2.6)	2 (11.1)	Ref		Ref	
Yes	240 (96.7)	224 (97.3)	16 (88.8)	0.18 (-0.11 to 0.48)	0.23	0.21 (0.03 to 1.14)	0.07

Possess livestock							
No	66 (26.6)	62 (26.9)	4 (22.2)	Ref		Ref	
Yes	182 (73.3)	168 (73.0)	14 (77.7)	0.01 (-0.10 to 0.13)	0.81	1.29 (0.40 to 4.07)	0.66
Registered under below poverty families (BPL)							
No	155 (62.5)	144 (62.6)	11 (61.1)	Ref		Ref	
Yes	93 (37.5)	86 (37.3)	7 (38.8)	0.08 (-0.02 to 0.19)	0.13	1.06 (0.39 to 2.85)	0.89
Sold any assets in past 12 months (assets such as land, house, gold, valuables sold prior to the study recruitment)							
No	204 (82.2)	187 (81.3)	17 (94.4)	Ref		Ref	
Yes	44 (17.7)	43 (18.7)	1 (5.5)	0.05 (-0.08 to 0.19)	0.42	0.25 (0.03 to 1.97)	0.19
Dietary preferences and pattern recorded at the time of recruitment							
Vegetarian	104 (41.9)	94 (40.8)	10 (55.5)	Ref		Ref	
Eggetarian	28 (11.2)	27 (11.7)	1 (5.5)	0.09 (-0.08 to 0.27)	0.31	0.34 (0.04 to 2.84)	0.32
Non-vegetarian	116 (46.7)	109 (47.3)	7 (38.8)	0.09 (-0.02 to 0.20)	0.11	0.60 (0.22 to 1.64)	0.32
Pure Milk							
≤ 2 times a week	134 (54.0)	120 (52.1)	14 (77.7)	Ref		Ref	
≥ 3 times a week	114 (45.9)	110 (47.8)	4 (22.2)	0.03 (-0.07 to 0.14)	0.51	0.31 (0.09 to 0.97)*	0.04
Green leafy vegetables							
≤ 2 times a week	140 (56.4)	126 (54.7)	14 (77.7)	Ref		Ref	
≥ 3 times a week	108 (43.5)	104 (45.2)	4 (22.2)	0.04 (-0.06 to 0.14)	0.45	0.34 (0.11 to 1.08)	0.06
Bean sprouts							
≤ 2 times a week	241 (97.1)	223 (96.9)	18 (100)	Ref		Omitted	-
≥ 3 times a week	7 (2.8)	7 (3.0)	0	0.04 (-0.28 to 0.36)	0.79	-	-
Pulses-lentils							
≤ 2 times a week	69 (27.8)	63 (27.3)	6 (33.3)	Ref		Ref	
≥ 3 times a week	179 (72.1)	167 (72.6)	12 (66.6)	0.07 (-0.04 to 0.19)	0.22	0.75 (0.27 to 2.09)	0.58
Fruits/juices							
≤ 2 times a week	134 (54.0)	121 (52.6)	13 (72.2)	Ref		Ref	
≥ 3 times a week	114 (45.9)	109 (47.3)	5 (27.7)	0.08 (-0.01 to 0.19)	0.10	0.42 (0.14 to 1.23)	0.11
Rice							
≤ 2 times a week	18 (7.2)	15 (6.5)	3 (16.6)	Ref		Ref	
≥ 3 times a week	230 (92.7)	215 (93.4)	15 (83.3)	0.03 (-0.16 to 0.24)	0.70	0.34 (0.09 to 1.33)	0.12

Eggs							
≤ 2 times a week	234 (94.3)	217 (94.3)	17 (94.4)	Ref		Ref	
≥ 3 times a week	14 (5.6)	13 (5.6)	1 (5.5)	0.07 (-0.15 to 0.30)	0.53	0.98 (0.12 to 7.69)	0.98
Chicken							
≤ 2 times a week	242 (97.5)	224 (97.3)	18 (100)	Ref		Omitted	-
≥ 3 times a week	6 (2.4)	6 (2.6)	0	0.31 (-0.02 to 0.66)	0.07	-	-
Goat meat							
≤ 2 times a week	247 (99.5)	229 (99.5)	18 (100)	Ref		Omitted	-
≥ 3 times a week	1 (0.5)	1 (0.4)	0	-0.39 (-1.23 to 0.44)	0.35	-	-
Cooking fuel							
Smoke free (LPG, electric range, kerosene)	47 (18.9)	44 (19.1)	3 (16.6)	Ref		Ref	
Causes smoke (wood/straw/animal dung)	201 (81.0)	186 (80.8)	15 (83.3)	-0.06 (-0.20 to 0.06)	0.33	1.18 (0.32 to 4.26)	0.79
Heard about anaemia and Haemoglobin (Hb)							
No	180 (72.5)	163 (70.8)	17 (94.4)	Ref		Ref	
Yes	68 (27.4)	67 (29.1)	1 (5.5)	0.07 (-0.04 to 0.19)	0.23	0.14 (0.01 to 1.09)	0.06
Checked Hb during current pregnancy							
No	106 (42.7)	100 (43.4)	6 (33.3)	Ref		Ref	
Yes	142 (57.2)	130 (56.5)	12 (66.6)	-0.08 (-0.19 to 0.02)	0.12	1.53 (0.55 to 4.24)	0.40
Knew recent Hb levels							
No	191 (77.0)	180 (78.2)	11 (61.1)	Ref		Ref	
Yes	57 (22.9)	50 (21.7)	7 (38.8)	-0.03 (-0.16 to 0.08)	0.55	2.29 (0.84 to 6.21)	0.10
Pregnancy registration at the time of recruitment							
Registered with government or private hospital	233 (93.9)	215 (93.4)	18 (100)	Ref		Omitted	-
Not registered with any hospital	15 (6.0)	15 (6.5)	0	0.03 (-0.19 to 0.25)	0.78	-	-

Table 15 footnotes:

- p<0.05, ** p<0.001

- Annual incomes expressed in GBP: Great Britain Pound (1 GBP= 100 Indian Rupees)
- Religion and categories are important component of Indian societal structure. Category represents ethnic background, which are mainly divided into open and reserved classes.
- For linear regression birth weight values were used as a continuous outcome, and binary category variable (low birth weight yes/no) is used in logistic regression. Ref indicates reference category.
- Low birth was defined as a birth weight less than 2.5 Kg (2500 grams) at birth.

Table 16: Adjusted regression results (multivariable model): individual risk factors associated with low birth weight) (N= 248 pregnant women)

Characteristics	Unadjusted linear regression β (95% CI) [birth weight in Kg]	Adjusted linear regression β (95% CI) [birth weight in Kg]	Unadjusted logistic regression OR (95% CI)	Adjusted logistic regression OR (95% CI)
Maternal age	0.00 (-0.01 to 0.01)	-0.01 (-0.03 to 0.01)	0.98 (0.83 to 1.16)	1.22 (0.08 to 0.96)
Mid-upper arm circumference of dominant hand (MUAC)				
< 22	Ref	Ref	Ref	Ref
≥ 22 cm and above	0.11 (-0.06 to 0.30)	0.12 (-0.06 to 0.31)	0.76 (0.16 to 3.55)	0.77 (0.11 to 1.24)
Consuming Iron Folic Acid supplements at the time of recruitment				
No	Ref	Ref	Ref	Ref
Yes	0.05 (-0.06 to 0.18)	0.06 (-0.08 to 0.20)	0.44 (0.16 to 1.21)	0.37 (0.11 to 1.24)
Gravidity	0.02 (-0.03 to 0.07)	0.06 (-0.00 to 0.14)	0.79 (0.44 to 1.41)	0.37 (0.16 to 0.83)
Diet recall (recent 7-days history) recorded at the time of recruitment				
Pure milk (not in the form of tea/coffee)				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.03 (-0.07 to 0.14)	0.04 (-0.07 to 0.15)	0.31 (0.09 to 0.97)*	0.22 (0.04 to 1.03)
Green leafy vegetables				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.04 (-0.06 to 0.14)	0.01 (-0.10 to 0.12)	0.34 (0.11 to 1.08)	0.56 (0.14 to 2.14)
Bean sprouts				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.04 (-0.28 to 0.36)	0.01 (-0.32 to 0.34)	Omitted	Omitted
Pulses-lentils				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.07 (-0.04 to 0.19)	0.05 (-0.06 to 0.18)	0.75 (0.27 to 2.09)	1.19 (0.34 to 4.12)
Fruits/Fruit juices				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.08 (-0.01 to 0.19)	0.07 (-0.03 to 0.18)	0.42 (0.14 to 1.23)	0.48 (0.14 to 1.62)

Rice				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.03 (-0.16 to 0.24)	-0.01 (-0.22 to 0.20)	0.34 (0.09 to 1.33)	0.34 (0.07 to 1.73)
Eggs				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.07 (-0.15 to 0.30)	0.04 (-0.20 to 0.29)	0.98 (0.12 to 7.69)	1.14 (0.07 to 16.70)
Chicken				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	0.31 (-0.02 to 0.66)	0.30 (-0.05 to 0.66)	Omitted	Omitted
Goat meat				
≤ 2 times a week	Ref	Ref	Ref	Ref
≥ 3 times a week	-0.39 (-1.23 to 0.44)	-0.57 (-1.47 to 0.32)	Omitted	Omitted
Education completed				
Primary school	Ref	Ref	Ref	Ref
Secondary school	0.00 (-0.12 to 0.12)	0.03 (-0.10 to 0.16)	0.78 (0.26 to 2.31)	0.83 (0.22 to 3.09)
≥ Higher secondary school	0.07 (-0.07 to 0.21)	0.07 (-0.08 to 0.23)	Omitted	Omitted
Consanguineous marriage				
No	Ref	Ref	Ref	Ref
Yes	-0.11 (-0.23 to -0.00)*	-0.12 (-0.25 to -0.00)*	3.13 (1.25 to 8.77)*	4.10 (1.24 to 13.41)*
Current pregnancy registration at health facility				
No	Ref	Ref	Ref	Ref
Yes	0.03 (-0.19 to 0.25)	0.03 (-0.21 to 0.28)	Omitted	Omitted
Religion and category				
Hindu open category	Ref	Ref	Ref	Ref
Hindu reserved category	0.01 (-0.09 to 0.12)	0.02 (-0.09 to 0.14)	1.40 (0.52 to 3.78)	0.83 (0.23 to 2.94)
Muslim	0.10 (-0.12 to 0.33)	0.15 (-0.10 to 0.41)	1.07 (0.12 to 9.20)	0.65 (0.04 to 10.75)
Annual income				
≤ 500 GBP	Ref	Ref	Ref	Ref
Between 501 and 1000 GBP	-0.05 (-0.16 to 0.05)	-0.04 (-0.16 to 0.07)	1.22 (0.45 to 3.22)	1.71 (0.50 to 5.78)
≥ 1001 GBP	0.06 (-0.15 to 0.27)	0.04 (-0.18 to 0.27)	Omitted	Omitted

Table 16 footnotes:

- * Indicates $p<0.05$. Birth weight is used as a continuous measure in linear regression, and in the logistic regression it is classified as a binary categorical variable (LBW and non-LBW).
- Birth weight unit used: kilograms (Kg). OR= Odds ratio, β=linear regression correlation coefficient, CI= confidence interval.

- Income 1 Great Britain Pound (GBP) was considered equivalent to 100 Indian Rupees.
- Maternal age and gravidity used as a continuous variable following linearity assessment.
- Ref indicates reference category.

4.4.5 Regression analyses summary

The following table summarised results from anaemia and low birth weight risk factor analysis (Table 17). Consanguineous marriage reported significant association with maternal anaemia and low birth weight. Access to government nurse was associated with lower risk of anaemia, but this did not remain significant on full adjustments.

Table 17: Summary of factors associated with maternal anaemia and low birth weight

Analysis	Risk factor	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
a. Individual risk factors associated with anaemia during pregnancy	Consanguineous marriage	2.21 (1.11 to 4.40)	2.41 (1.16 to 5.01)*[a]
b. Village level risk factor associated with anaemia during pregnancy	Daily access to government nurse ^	0.48 (0.25 to 0.93)	0.48 (0.19 to 1.19)* [b]
c. Individual risk factors associated with low birth weight	Consanguineous marriage	3.13 (1.25 to 8.77)	4.10 (1.25 to 13.41)*[a]

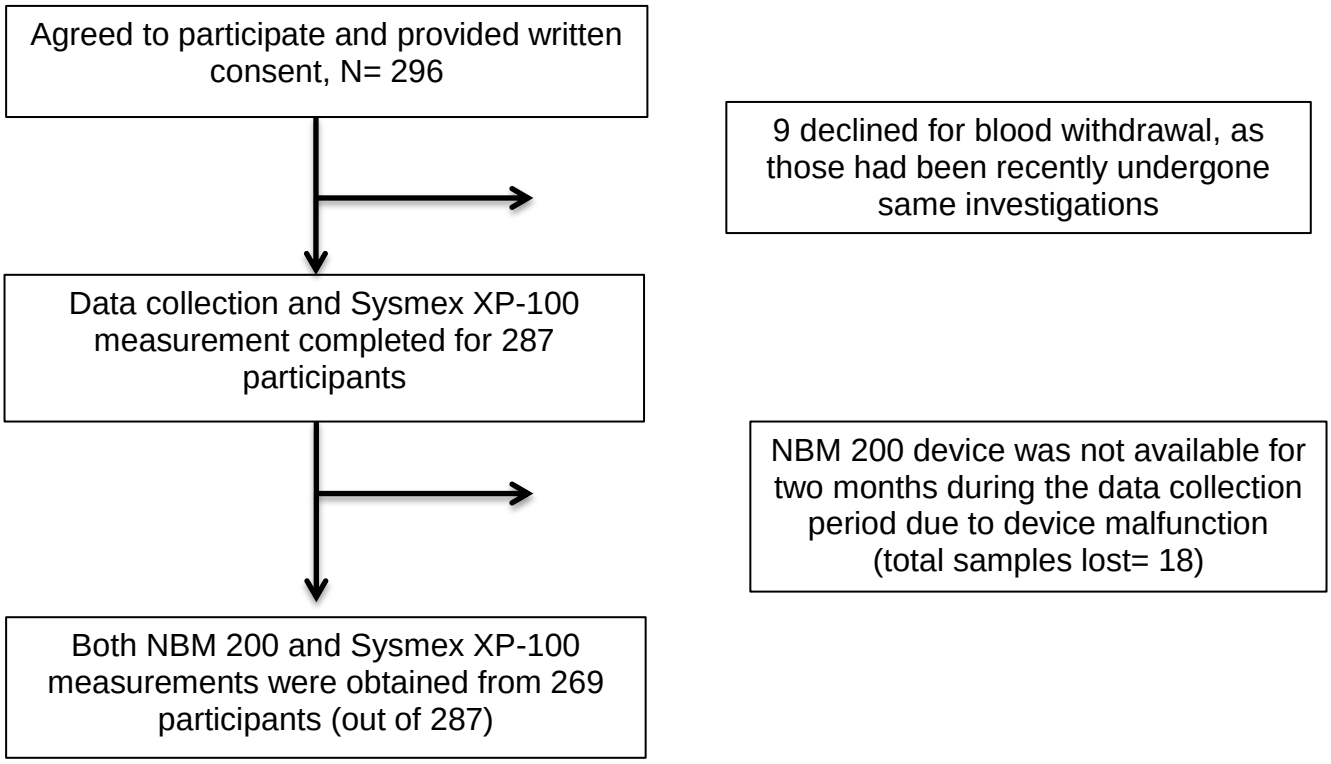
Table 16 footnotes:

- * Indicates p values <0.05. OR= Odds ratio, CI= confidence intervals.
- **[a]** Indicates adjusted results for maternal age, MUAC, IFA, gravidity, 7-day dietary recall, maternal education, religion, pregnancy registration with health facility and income.
- **[b]** Indicates adjusted results for sanitation facilities (village water supplies, toilets), health facilities (government health centre, ASHA, private doctor, ANC care, Hb testing services, private doctor availability, travel time to hospital).
- **^** Access to government nurse was the only significant factors in the unadjusted analysis (village level risk factor group), however on full adjustments no significant results were observed.

4.4.6 NBM 200 validation among pregnant women population

The NBM 200 device was not available due to technical reasons for three months during the study period, thus non-invasive Hb measurements were obtained from 269 pregnant women of 287 participants (Figure 24). The validation analysis is based on 269-paired measurements.

Figure 24: Flow chart of process implemented to obtain NBM 200 and Sysmex XP-100 measurements



Hb estimated by the Sysmex XP-100 (gold standard) ranged from 5.5 g/dl to 14.1 g/dl (mean: 10.0 g/dl), while the Hb estimated by the NBM 200 ranged from 9.5 g/dl to 14.6 g/dl (mean: 11.9 g/dl). Measurements from the reference method were normally distributed, but those generated by NBM 200 showed a plateau like presentation (Figure 25). The Sysmex XP-100 analyser (gold standard) results reported a high prevalence of

anaemia (77.3%) in the study population with 5 (1.9%) individuals classified with severe anaemia (Table 18). The NBM 200 sensor measurements classified 27.9% of the study population with anaemia and none with severe anaemia.

Figure 25: Histogram of Hb measurements obtained using NBM 200 (N=269, Hb in g/dl)

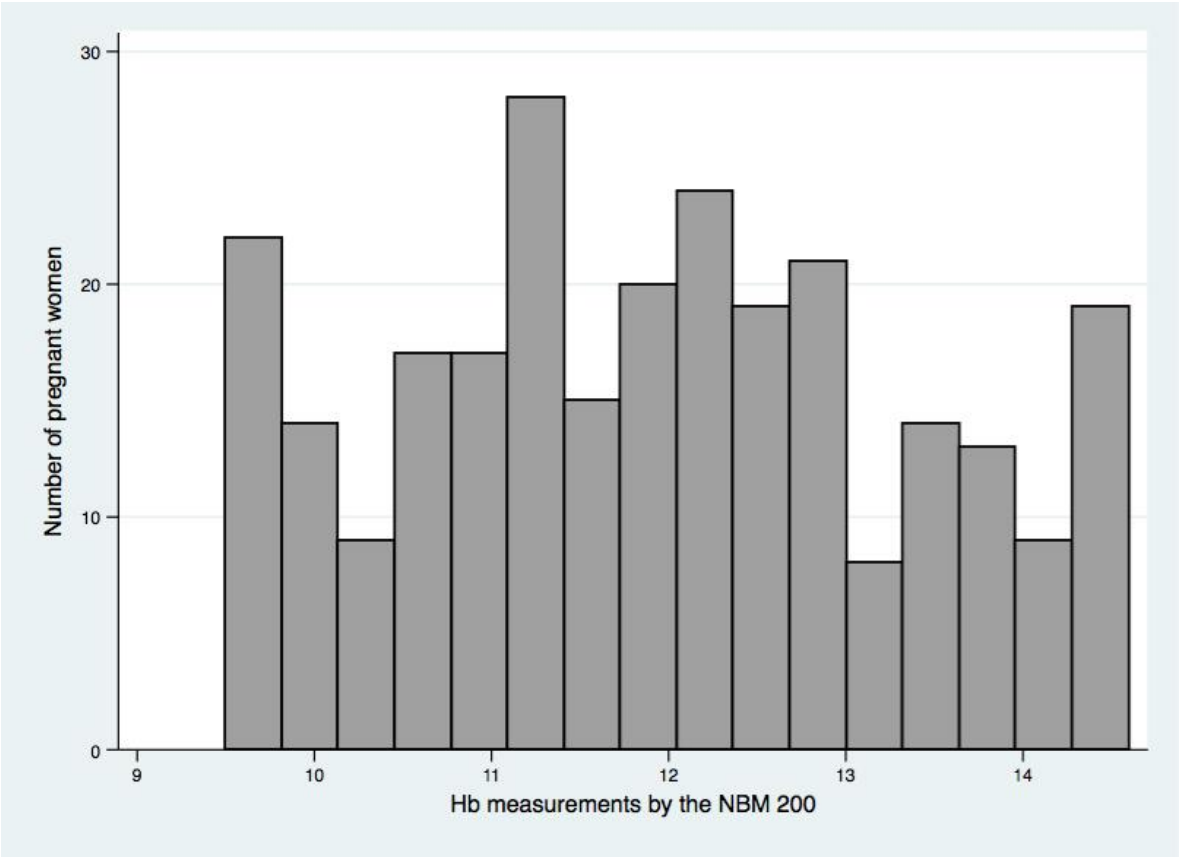
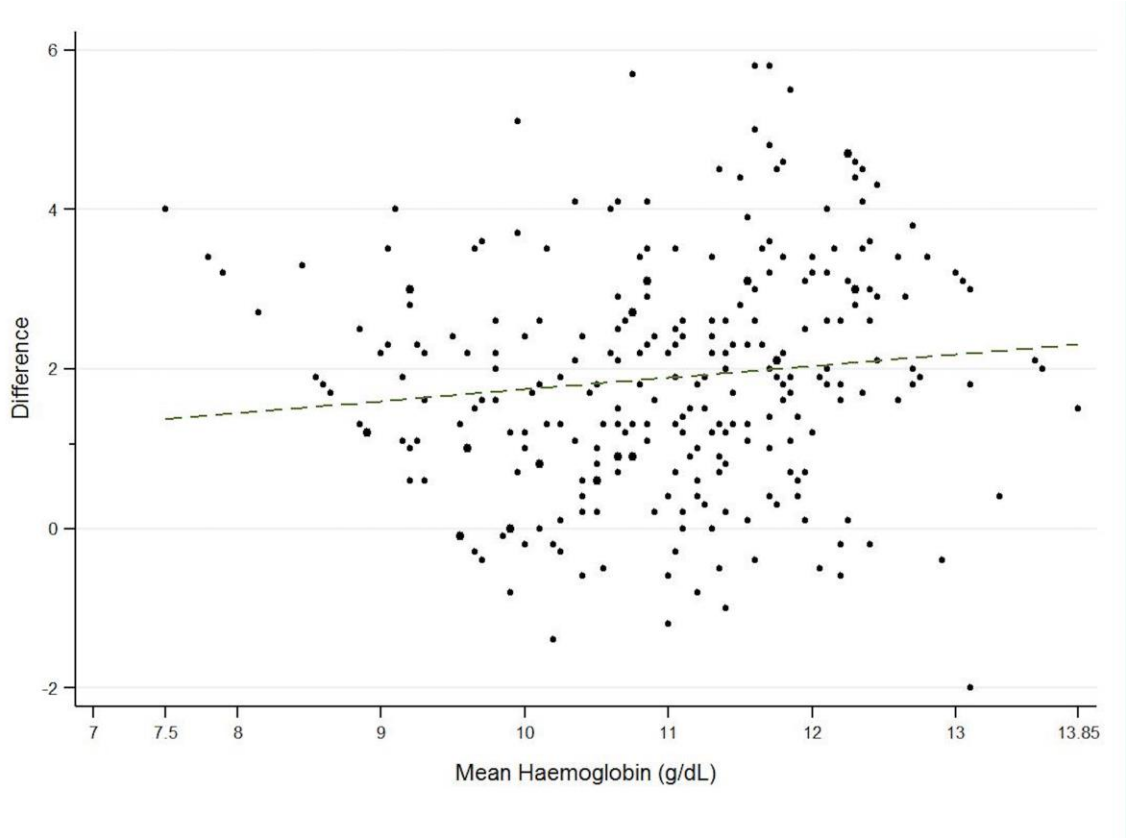


Table 18: Cases of severe anaemia, which were measured as moderate anaemia by the non-invasive sensor

No	Hb measurement by Sysmex XP-100 haematology analyser	Hb measurement by NBM 200 sensor for the same participant
1	5.5 g/dl	9.5 g/dl
2	6.1 g/dl	9.5 g/dl
3	6.3 g/dl	9.5 g/dl
4	6.8 g/dl	10.1 g/dl
5	6.8 g/dl	9.5 g/dl

The Bland-Altman plot is presented in Figure 26. A comparison of measurements from the two Hb estimation methods yielded a mean difference of -1.9 g/dl suggesting a systematic overestimation of Hb using the NBM 200. In 11.2% cases the NBM 200 yielded Hb measurements that were equal to or marginally higher than the measurements from the Sysmex XP-100.

Figure 26: Bland Altman Plot of Sysmex XP-100 and NBM 200 (N=269)



A statistically significant, moderately positive correlation was observed between NBM 200 and Sysmex XP-100 measurements (Spearman's correlation coefficient r_s= 0.4, p<0.001). Diagnostic accuracy for the NBM 200 for anaemia were as follows: sensitivity 33.7% (95% CI: 27.3%, 40.5%), specificity 91.8% (95% CI: 81.9%, 97.3%), positive predictive value 93.3% (95% CI: 85.1%, 97.8%), and negative predictive value 28.9%

(95% CI: 22.6%, 35.8%). The area under the receiver operating characteristic curve was 0.63 (95% CI: 0.58, 0.67).

4.5 Discussion

4.5.1 Principal findings

A very high prevalence of anaemia was documented in pregnant women from rural Maharashtra. Results showed that in the majority of participants MCV was lower than the first trimester threshold (Mean: 80.5 fl/cell, SD: 7.22, Range 53.4 to 93.8), indicating a combination of deficiencies including iron, vitamin B12 and folic acid. Our findings reported that consanguineous marriage was associated with maternal anaemia and LBW. Local village access to government nurse showed lower likelihood of anaemia.

4.5.2 Strengths and limitations

Our study has several strengths. To our knowledge this is the first community-based study where prospectively collected data are presented from a representative population of pregnant women in a rural setting of Maharashtra state. Our ability to conduct systematic recruitment, blood investigations, quality controls, and follow-ups are major strengths of the study. The study population was drawn from marginalised and difficult to access areas where comparatively few healthcare facilities are available, and thus evaluating non-invasive portable technology in geographically remote areas holds importance. The non-invasive readings were obtained in accordance with the manufacturer's guidelines, and none of our subjects had thumb deformity, infection, ulcerations, oedema, or skin colourants such as henna (locally known as Mehndi) [79].

It is important to consider that our analysis has a number of limitations. These are explained in turn below:

- Sample size and generalisability
- Chance
- Bias
- Confounding
- Limitations related with blood investigations

Sample size and generalisability: The key limitation is no prior sample size calculation as this was a feasibility study with a fixed number of study participants due to limited timeframe and financial resources. The study observations are applicable to Maharashtra state, and not necessarily generalisable to other regions/states in India. It was not possible to compare our estimates to any baseline pregnancy data on anaemia, as there is no information recorded in this rural population.

Chance: The statistically non-significant association observed between a number of studied risk factors and anaemia may be due to the small sample size, and thus findings should be interpreted with caution, guided by the precision of the estimates as demonstrated by the 95% confidence intervals.

Bias: Information on dietary intake, anaemia history, and medical history related covariates are subject to bias that is likely to be non-differential. Information on other variables collected may have been affected by reporting bias such as variables related with socioeconomic status, household resources, and family income. Every effort was made to verify such information with the HMF's village healthcare workers during the data collection. Subsequently, information on health and social data were collected prior to anaemia investigation, when pregnant women and also data

collectors were not aware about the anaemia status. Thus, our study is less likely to have any differential bias.

Confounding: While considerable effort was put into collecting data on all relevant covariates related to pregnancy, there may be other unknown factors associated with maternal anaemia in this study population. Other factors not collected in our study that may have an association with low birth weight such as genetic aspects linked through the consanguineous relationship is important to consider; however this was beyond the scope of this research. Though every attempt was made to minimise various biases, we acknowledge that some bias may remain due to our study approach.

Limitations related with blood investigations: There was no availability of haemoglobin measurements of study participants prior to conception, which would have allowed the identification of the start of anaemia. Additional Hb follow-up across pregnancy may have offered useful data to investigate anaemia prevalence in each trimester, and its risk factors. We were unable to conduct additional investigations due to limited infrastructural and financial resources such as serum iron, vitamins and folic acid. Similarly, due to the restricted study duration and human resource, data collection (particularly blood investigations) was not feasible across three trimesters.

4.5.3 Synthesis

Risk factors of maternal anaemia

In the Indian national family health surveys (NFHS-2 and NFHS-3), pregnant women from all stages/trimesters were included in the anaemia assessment [117] . Results from the NFHS 2 (1998-99, N= 5619 pregnancies from 28 Indian states) reported an overall prevalence of anaemia of 47%, with mainly mild and moderate anaemic cases [103].

Findings from the NFHS 3 (2005-06) reported an increase in anaemia prevalence (59%, N= 6028 pregnant women)[101]. Our study reported a higher prevalence compared to both NFHSs. These surveys showed that the prevalence varied across and within Indian states suggesting the influence of local lifestyles and cultural norms, individual medical conditions, infrastructure, and healthcare services as explained earlier. For example, a study in a remote north-eastern region (Assam State) reported a prevalence of 90% [104]. Though our study from Central India reported a high anaemia prevalence in pregnancy, no such difference was seen in subsequent birth weight among anaemia and non-anaemic mothers. Published studies from Maharashtra state showed a 30% to 65% anaemia prevalence in pregnancy with higher prevalence in rural regions [13,97–99]. Studies from South India found 55% to 60% anaemia among pregnant women in a region where consanguineous marriage is a common practice [118].

A recent Indian Human Development Survey reported a national prevalence of 16% of consanguineous marriages, with a higher occurrence in Maharashtra state (28%) [118]. In such relationships, the maternal cousin (maternal uncle's son) is the most preferred partner in our study area, and these marriages are favoured due to local cultural and economic reasons. Currently there is no other published evidence on the association between consanguinity and maternal anaemia and this needs further independent inquiry.

Analysis of the village level data showed lower likelihood of maternal anaemia associated with regular access to government nurses who conduct routine ANC checks-ups, provide vaccinations and free IFA supplies. In villages where nurses were permanently stationed, improved access to such services, compared to remote villages receiving monthly visits could explain this association. Alternatively, there are many

environmental factors that may contribute to residual confounding. This is the first analysis where village factors were analysed, and to our knowledge, no such analysis is published from other parts of India to compare our findings.

Risk factors of low birth weight

Our study did not report any association of socioeconomic status with maternal anaemia and LBW. We observed that anaemia was widespread in our study area involving women from all social classes. The prevalence of LBW was low (7%) compared to the national survey findings (20%) [13]. This may be due to the small sample size in our study.

Findings from one of the oldest Indian studies showed that on an average babies born to consanguineous participants weighed 0.25 Kg less than babies from non-consanguineous relationships [119]. However, this observation was limited to poor socioeconomic groups when compared with participants from higher socioeconomic class. Findings from the prospective cohort of pregnant women (N=601) in rural regions of Karnataka state showed an increase in LBW incidence among consanguineous couples (p=0.04) [120]. The study also showed higher risks of stillbirth associated with consanguinity. A recent 2013 cross-sectional analysis from Karnataka state reported higher risk of LBW with consanguineous marriages [121]. Findings from these three Indian studies are in agreement with our results. Similarly, studies from Lebanon, Qatar, Pakistan and Jordan have reported higher risks of LBW in consanguineous relationship [122–125]. In consanguineous couples, a study from Lebanon reported a significant 1.8% decrease in birth weight [122], full term babies born in Qatar were nearly twice as likely to be LBW [123], the study from Pakistan showed 14% higher risk of small babies [124].

4.5.4 NBM 200 device validation

The validation involving women during pregnancy found that haemoglobin measurements obtained from the NBM 200 were generally higher than the measurements obtained from the Sysmex XP-100 (gold standard). Consequently, the NBM 200 grossly underestimated the anaemia prevalence in our study population (sensitivity of 33.7%). We also identified possible patient safety concerns with the use of the NBM 200, considering all the severe anaemia cases in our study participants were misclassified as having moderate anaemia and would not have received appropriate clinical intervention had this method been used alone (Table 18).

Our study findings are in agreement with those from a study conducted at a blood centre in Seoul, Korea by Kim et al [94], where the NBM 200 Hb measurements tended to be higher than the LH500 automated hematology analyser estimates (Beckman Coulter Inc., Brea, CA, USA). The sensitivity (38.6%) and specificity (93.6%) outcomes were very similar to our findings, and indicated that the NBM 200 failed to detect more than half of the ineligible blood donors. Though the correlation between these two techniques was satisfactory (r_s= 0.86), the strong agreement may not be the criteria for donor selection, as accurate blood parameters are prerequisites to prevent any ineligible donor selection. Similarly, an Italian study (n= 3995 donors) showed a low sensitivity using NBM 200 (36.03% in men group and 45.76% among women) on comparison with the gold standard Beckman Coulter's AcT-5 diff AL (Beckman Coulter Inc., Brea, CA, USA) [126].

However, our findings are in contrast to a recent study involving blood donors (N= 485, 94% men) from North India that reported 71.7% sensitivity for the NBM 200 when

compared with the Sysmex KX-21 analyser (Sysmex Corporation, Japan) (Table 19) [127]. Despite the high sensitivity, the Bland Altman limits of agreement were wide (Upper limit 2.09 g/dl, and Lower limit -3.39) with poor correlation (r_s 0.43) between the two Hb estimation methods. About 45.5% of ineligible donors were not detected in the Indian study [127], similar to the study by Kim et al [94]. A second study from Northwest India involving 200 blood donors (96% men) showed high sensitivity (96.36%) when compared with the Sysmex KX-21 analyser (Sysmex America Inc., Lincolnshire) [128]. The results by NBM 200 showed wide variation on assessing correlation with the gold standard; however the mean of difference was non-significant [128]. Belardinelli and colleagues also reported high sensitivity (98%) and specificity (97%) in a similar study population [129]. It is worth noting however, that all the patients in their study had Hb values above 13 g/dl (as ascertained using the Beckman Coulter AcT-5 diff cell counter) and unlikely to be representative of the general population.

A study involving pregnant women from Israel (N=63) reported Hb in the range 6.9 g/dl to 13.9 g/dl by the gold standard (LH750 analyser, Beckman Coulter Inc., Brea, CA, USA), which was lower than the range reported by the NBM 200 (7.7 g/dl to 14 g/dl) [130]. The Bland Altman analysis showed wide limits of agreement (-1.59 to 1.79) with a standard deviation error of 0.86 g/dl. The study concluded that the sensor measures haemoglobin accurately mainly based on a strong correlation, however, correlation measures association and not agreement [78,94]. Secondly, the range of Hb values obtained by the gold standard and NBM 200 clearly indicate that the NBM 200 missed some severe anaemia cases similar to our experience [130]. Lastly, the study involved pregnant women from all trimesters (gestational age: average 35.9 weeks, range 13 to 41 weeks), where Hb is likely to vary [131], while our findings are based on larger sample

size (N=269) between a fixed gestational period (3 to 5 months of pregnancy) and included a more detailed diagnostic analysis reporting the Bland Altman agreement, sensitivity, specificity, NPV, PPV and AUROC values.

Previously published studies of the NBM 200 sensor (Table 19) have been mostly conducted in prospective blood donors [79,126–129], in whom Hb is likely to be higher than the general population. Moreover, the two Indian studies outlined previously, had fewer than 7% women participants [127,128]. A report from Indian National Family Health Survey 2005-06 reported a 24% anaemia prevalence in men (aged 15-49 years), which was much lower than in women (55% prevalence, 15-49 years) [132]. Further, anaemia prevalence in pregnant women was about 65%. Based on our results, the NBM 200 underestimates anaemia prevalence; therefore, the validation of the non-invasive technology predominantly in male blood donors could explain the different findings. The study by Singh et al. had a prevalence of 2.3% anaemic cases identified by the gold standard [127], and the study by Malukani et al. had only 17% anaemic cases [128]as compared to our study with 77% anaemic cases, suggesting sampling variability [75]. Studies from Italy, Germany and France involved fairly equal number of men and women reporting to blood donation centres, but anaemia prevalence is much lower in these countries compared to India. The German study reported a mean haemoglobin value of 13.4 g/dl (SD: 0.93) in women (using gold standard, Sysmex KX-21) [79], while in our study settings in rural India mean haemoglobin was 10.0 g/dl (using gold standard, Sysmex XP-100). Similarly, the mean haemoglobin in the French study was 13.2 g/dl (95% CI: 11.9, 14.3) [133], and in the Italian study the boxplot indicated a mean Hb of 14.0 g/dl [129].

A systematic review and random effects meta-analysis by Kim et al of non-invasive Hb sensor technologies (Radical 7, NBM 200, Pronto 7 and NBM 200MP) found that the pooled mean difference and SD were 0.10, + - 1.37 g/dl respectively (95% CI: -2.59, 2.80, I^2 = 95.9% for mean difference and I^2 =95.0% for SD) [75]. The review concluded that because of the wide limits of agreement with reference methods, clinical decisions based on non-invasive devices should be made cautiously [75]. All studies included in the review were hospital-based, and none of the studies were from developing countries where anaemia prevalence is typically much higher.

Table 19: Published studies conducted to validate NBM 200 sensor

First author, year of publication	Study settings	Country, population and sample size	Method of comparison and gold standard used	NBM 200 performance
Gayat Etienne et al, 2012	Emergency department, Foch university hospital, Suresnes, France.	France Patients reporting to emergency department n=569 and for NBM 200 n= 297 (men 157 and women 140) Males 52.9% Females 47.1% Data analysed for 270 patients.	Descriptive data, student t test, Bland Altman analysis, Intra-class correlation, Multivariate linear regression to identify variables associated with bias. ADVIA 2120 (Siemens Medical Solutions Diagnostics, Zurich, Switzerland).	Bias: 0.21 g/dl (95% CI: 0.02, 0.39) Bland Altman analysis Upper limit of agreement: 3.42 g/dl (95% CI: 3.10, 3.74) Lower limit of agreement: -3.01 g/dl (95% CI: -3.32, -2.69) Intra-class correlation coefficient: 0.69 (95% CI: 0.62, 0.75) Coefficient of variation (CV): 5.9% True values of haemoglobin and perfusion index were independently associated with NBM 200. Despite the limited bias, wide level of agreement was found, thus clinical usefulness is debatable.
Singh Abhay et al, 2015	Blood donation centre in tertiary care institute, India.	India Blood donors n= 534, and 485 included in the analysis for NBM 200 (men 455, women 30) n= 485	Bland Altman analysis, intra-class correlation coefficient, Specificity, Sensitivity, Negative predictive value (NPV) and Positive predictive value (PPV).	Mean Hb by NBM 200:14.8 g/dl Mean Hb by the gold standard: 14.1 g/dl SD for NBM 200 > 2.0 g/dl, on comparing with gold standard. Bland Altman Agreement ULA: 2.09 g/dl (95% CI: 1.88, 2.30) LLA : - 3.39 g/dl (95% CI : - 3.61, - 3.19)

		Males 94% Females 6%	Sysmex KX-21 (Sysmex Corporation, Japan).	Bias: -0.66 g/dl (95% CI: -0.78, -0.53) Sensitivity 71.7%, Specificity 79.5%, NPV 95.8%, PPV 30.2% Intra-class correlation: 0.43 (95% CI: 0.27, 0.55) NBM 200 did not detect 45.5% of ineligible donors. NBM 200 has benefit of pain elimination but carries a substantial possibility of ineligible donor selection.
Malukani Pankaj et al, 2014	Blood donation centre in tertiary care hospital, Ahmedabad, Gujarat, India.	India Blood donors n= 200 (men 192, women 8) Males 96% Females 4%	Specificity, Sensitivity, Negative predictive value and Positive predictive value. Sysmex KX 21 (Sysmex America Inc., Lincolnshire).	Mean Hb by NBM 200: 13.5 g/dl (SD 1.4) Sensitivity 96.36%, Specificity 91.43%, NPV 84.21%, PPV 98.15% Likelihood ratio 8.22 The results by NBM 200 showed wide variation when compared with the gold standard, but the mean of difference was non-significant.
Hadar Eran et al, 2012	Women's hospital, Rabin Medical centre, Petah Tikva, Israel.	Israel Pregnant women n= 63 Average age 31.3 years (range 21 to 25) Average gestational age	Bland Altman analysis LH750 automated hematology analyser (Beckman Coulter Inc., Brea, CA, USA).	Hb Range obtained by NBM 200: 7.7 g/dl to 14.0 g/dl SD 0.86 g/dl Bland Altman Agreement: -1.59 g/dl to 1.79 g/dl Correlation coefficient: 0.82 Mean absolute error: 0.71 g/dl Hb Range obtained by gold standard: 6.9 g/dl to 13.9 g/dl Study demonstrates good correlation with gold standard

		35.9 weeks (range 13 to 41 weeks)		and NBM 200 measure Hb accurately.
Sergey Ardin et al, 2015	Blood donation centre of the Institute for Transfusion Medicine of the University Hospital of Cologne, Germany.	Germany Blood donors Donors age mean 36 years (SD 14) n= 351, and for NBM 200 n= 120 (through phase 1 of the study, 78 men and 42 women). Men 65% Women 35%	Bland Altman analysis Sysmex KX-21n, (Sysmex Europe GmbH, Norderstedt, Germany).	SD for NBM 200 1.14 g/dl, on comparing with gold standard Bland Altman Agreement: -2.35 g/dl to 2.11 g/dl Bias: -0.12 g/dl False rejection of seven donors, however no correspondent unacceptable classification occurred. NBM 200 obtained lower haemoglobin levels in women.
Kim Moon Jung et al, 2013	Two blood donation sites affiliated to the Hanmaum Blood centre, Gwacheon, Korea.	Korea Blood donors n= 506 (men 291, women 215) Males 57.5% Females 42.4%	Bland Altman analysis, Specificity and Sensitivity. LH500 automated hematology analyser (Beckman Coulter Inc., Brea, CA, USA).	Mean Hb by NBM 200: 14.1 g/dl Intra-class correlation between NBM 200 and LH500: 0.86 NBM 200 Hb < 12.0 g/dl in 37 participants (mean 12.4, SD 1.2) Hb = 12.0 to 12.4 g/dl in 18 participants (mean 12.8, SD 1.1) Hb ≥ 12.5 g/dl in 451 participants (mean 14.4, SD 1.2) Bland Altman plot showed 2 SD difference in haemoglobin estimations between LH500 and NBM 200, Hb > 2.0 g/dl.

				Whole blood Sensitivity 38.6% (95% CI: 28.1, 50.3) Specificity 93.6% (95% CI: 90.9, 95.5) Apheresis Sensitivity 37.8% (95% CI: 24.1, 53.9) Specificity 94.0% (95% CI: 91.5, 95.8) Out of 70 ineligible donors (Hb < 12.5 g/dl) according to the gold standard (LH500), 43 donors (61.45%) were considered eligible by the NBM 200.
Paglirao Pasqualepaolo et al, 2014	Immunohematology and Transfusion Medicine Collection site of S. Orsola Malpighi Hospital, Bologna, Italy.	Italy Blood donors *Data from year 2012* NBM 200 (n) Men= 2945 Women=1021 *Data from year 2013 (n)* NBM 200 Men= 2895 Women=1100	Specificity, Sensitivity, Negative predictive value and Positive predictive value. Donors were classified into fit and unfit sections. Hematocytometry tests as a reference in year 2012. Beckman Coulter's AcT-5 diff AL (Beckman Coulter Inc., Brea, CA, USA) as a gold standard in year 2013.	*2012: NBM 200 and Hematocytometry test* Women (n= 1021) Sensitivity 32.34%, Specificity 95.12%, NPV 85.15% and PPV 61.90% Men (n= 2945) Specificity 98.89%, Sensitivity 14.47% NPV 95.30% and PPV 42.59% *2013: NBM 200 + targeted venous sample (gold standard, Beckman Coulter)* Women (n= 1100) Sensitivity 45.76%, Specificity 99.24%, NPV 90.51% and PPV 92.05% Men (n= 2895) Sensitivity 36.03%, Specificity 99.93%, NPV 96.94% and PPV 96.08%

				Compared to non-invasive techniques, a venous sample improves donor selection.
Belardinelli A et al, 2013	Immunohematology and Transfusion Medicine Collection site of S. Orsola Malpighi Hospital, Bologna, Italy.	Italy Blood donors n= 445 (men 296, women 149) Males 66.5% Females 33.4%	Bland Altman analysis, Specificity and Sensitivity. Beckman Coulter's AcT-5 diff AL (Beckman Coulter Inc., Brea, CA, USA).	Bland Altman Agreement: -1.64 g/dl to 2.21 g/dl Bias: 0.29 g/dl SD: 0.98 g/dl Percentage of donors screened correctly by NBM 200: 88% Sensitivity 98%, Specificity 97% Non-invasive technique does not replicate results of venous sample, and compared to finger-prick (HamoCue), the NBM 200 had low sensitivity and specificity.

4.5.5 Conclusion

Anaemia prevalence was very high in pregnant women population in rural Maharashtra. Consanguineous marriage was identified as a potential risk factor associated with maternal anaemia and low birth weight. Though village level risk factor assessment did not report any significant association in the adjusted analysis, further research is necessary about impact of community resources on maternal anaemia.

The NBM 200 validation study reported Hb overestimation suggesting the need to improve the technology substantially to provide accurate readings in line with the accepted gold standard, before it can be implemented in a rural Indian population. As most studies, including our own, suggest a systematic overestimation by the NBM 200, it may be possible for the manufacturer to improve the performance by recalibrating the sensor or algorithms that process the data collected. If the melanin content of the skin interferes with Hb readings, then this would need further consideration. Further field-based research in developing countries following improvements to the sensor is required, before advocating the adoption of non-invasive technology for haemoglobin measurement and anaemia diagnosis.

Chapter 5. Maharashtra birth registry: Longitudinal study

5.1 Introduction

This chapter describes the birth registry component of the thesis. The initial part will provide brief information on the global status of preference to son, followed by societal and cultural reasons for such preference in India. Major events contributing to the development of female foeticide prevention legislations in India are outlined in the second section. Current evidence gaps and purpose of this research are also described. Methods will inform the data collection and analysis strategy, followed by results and discussion. Manuscripts developed from this chapter are mentioned in the appendix 12 (list of publications).

5.2 Background on female foeticide

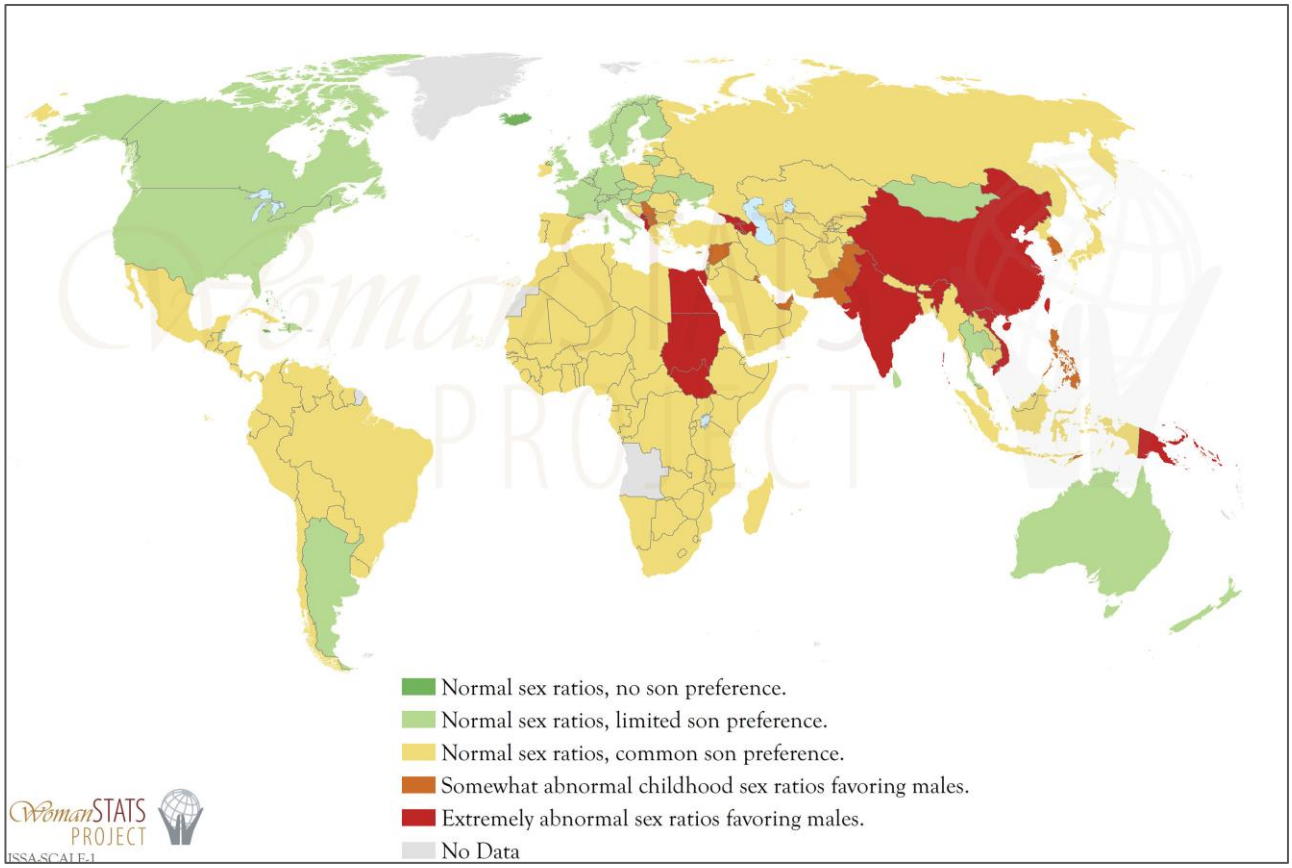
Societal preference for male offspring is observed in a number of countries, and may be a consequence of cultural and economic factors [134]. This is widely seen in Asian countries such as India, China, Republic of Korea, Vietnam and Pakistan [134,135]. Similar inclination is also observed in African countries include Egypt, Sudan, Morocco and Tunisia [134,135].

Following, the introduction of non-invasive diagnostic technologies mainly the ultrasound (USG) scanning, sex identification during pregnancy became feasible [136]. The device permits sex determination of the foetus from as early as 12 weeks of gestation, thus providing the possibility of sex-specific abortion once this information is known. A recent report from the United Nation suggested that nearly 117 million women are missing from Asia as a result of female foeticide [134]. The sex selective

abortion of female foetuses is classified as a gender based discrimination leading to severe societal issues such as imbalance sex ratios. The major impact of the skewed sex ratio is increase in violence against women, and human trafficking, therefore this is a serious public health concern [135,137].

The global sex ratio is 1.05; however in many countries the sex ratio at birth is skewed due to higher number of male births compared to female births (Figure 27).

Figure 27: Preference to sons- Global picture



Source: The figure is obtained from the Women Stats Project

Figure 27 footnotes: Countries indicated in red have extremely high preference to male offspring and have skewed sex ratios at birth, includes, India, China, Vietnam, Korea, Egypt, Sudan.

5.3 Foeticide issue in India

Sex specific foeticide is a growing problem in India, where the male: female sex ratio at live birth was approximately 1.16 in 2011, as opposed to a global sex ratio of 1.05 [138]. Causes for preference for male offspring in India are well documented, and these are embedded within the cultural and societal structure of the country [139]. The existing gender inequality and views on masculinity are one of the reasons for such preference (Table 20) [135,140]. Initially, it was thought that the inclination would exist in rural areas and commonly in poor communities. However recent studies showed that the issue epidemic, and observed across the country, irrespective of change in socioeconomic status, education and location [139,141]. The table outlined below presents some common intentions behind the strong preference to sons over daughters in Indian setting.

Table 20: Myths about male and female offspring in Indian communities [139,142–144]

Male offspring	Female offspring
Male child is considered stronger and a sign of prosperity.	Females are considered weak, and a cause of economic burden on a family.
Investing in son is considered productive as son could look after parents in old age.	After marriage female migrates to partner's house, thus parents are less inclined in supporting girl's education and general development.
Son receives money (generally in cash and gold) from his in-laws during marriage (known as Dowry), thus son is considered as a source of income.	Female births are thought to be a problem that results in costs for marriage and such ceremonies demands money (financial loss).
Son is less likely in need of protection in the society.	Female needs more protection in the society to ensure their safety.

5.4. Legislation developments in India

In India, ultrasonography (USG) devices were introduced in early 1970s. The device was initially available in major cities such as Mumbai, and later the technology was made available in smaller cities.

There was no legislation to regulate the use of USG devices in 1970s, which was new for both, radiologists and also for the government health department. During this time period, 'Abortion Act (1971)' was introduced by the government to reduce illegal and unsafe abortions with a primary objective to reduce maternal mortality [145]. This law granted permission to conduct abortion in a healthcare setting by a qualified medical doctor up to 20 weeks old pregnancy.

Importantly, the access to ultrasonography devices increased rapidly in the same time period (1970 to 1980), and the application to detect female foeticide was noticed [146,147]. Medical doctors with a licence of the 'Abortion Act' (also known as 'MTP'-Medical Termination of Pregnancy) initiated USG services for sex determination. This was documented by Dr Kulkarni in 1985, where his two years of research showed 200 cases of abortions in Karnataka state of India, which were performed under the 'Abortion Act' licence [147].

Social movements in India in alliance with human rights organisations to prevent female foeticide were started around 1978, which was further strengthened following findings by Dr Kulkarni. As a result, the alliance demanded legislations in the country to prevent female foeticide. Amartya Sen (an Indian Nobel Prize Winner) coined an important term 'missing women' in late 1980s, which attracted international attention

strengthening the Indian movement. The advocacy by various institutes across the country developed a pressure on the national government primarily after the 1991 census data, which showed reduction in female births compared to 1971 and 1981 records [22,138].

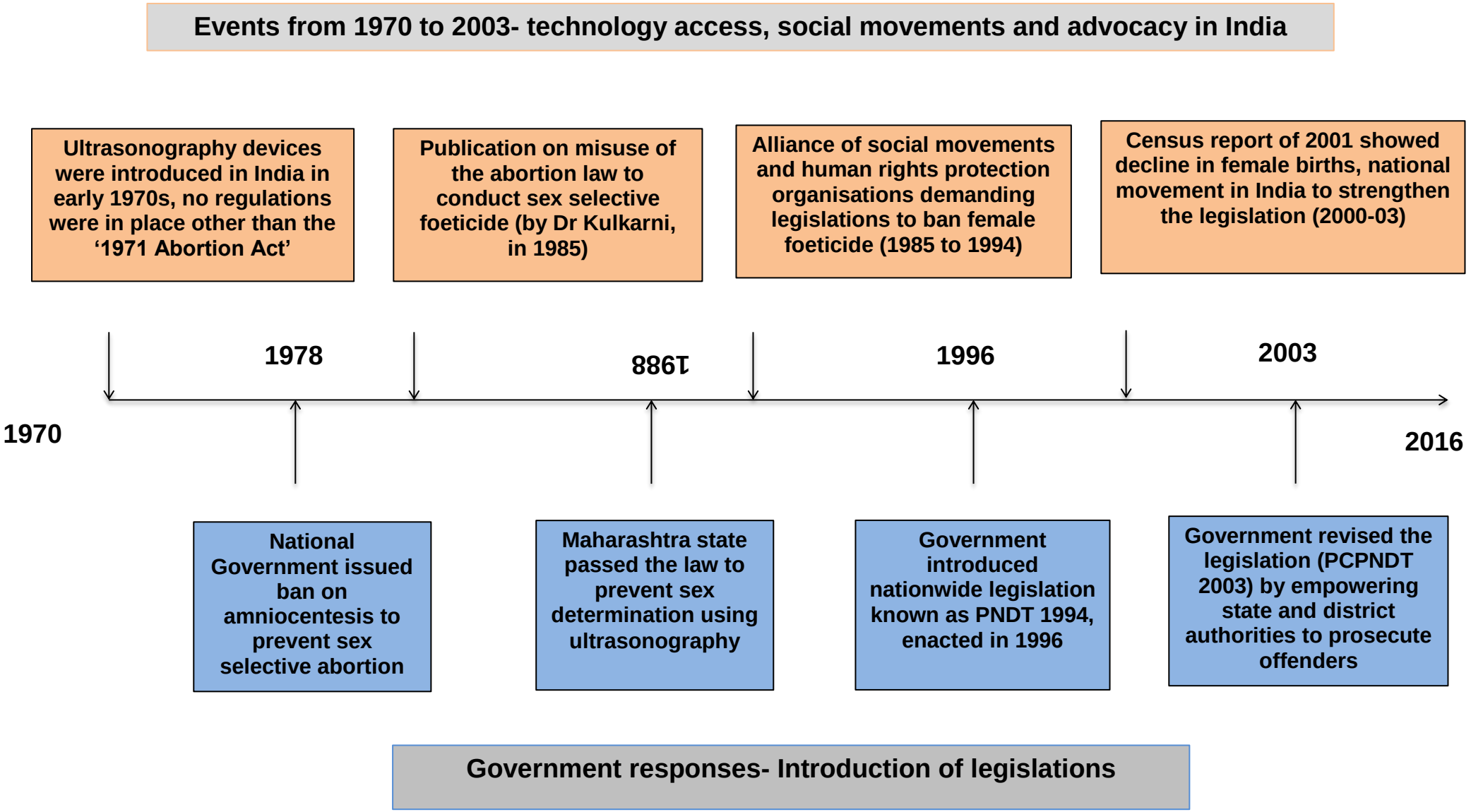
In response the national government passed the legislation (PNDT- The Pre-natal Diagnostic Techniques Act 1994) making determination of fetal sex illegal, which became active on the 1st January 1996 [148]. However the implementation of the law was poor [147].

The 2001 census record showed further decline in the sex ratio. As a consequence national and international organisation initiated a strong advocacy platform to strengthen monitoring of the legislation. In response, the government amended the PNDT Act that subsequently enforced on 14th February 2003 (known as PCPNDT 2003) [149]. The series of outlined events are presented in Figure 28.

According to the PCPNDT 2003 (Pre-conception and Pre-natal Diagnostic Techniques), each district across India, established authorities to conduct regular inspections of USG centres to examine medical record, identify discrepancies (female foeticide incident), and file prosecution order against the doctor and a family of the offender [149,150]. Each state developed a dedicated unit to monitor purchase, installation and the use of USG devices, known as PCPNDT Cell [150]. The amendment provided power to district authorities and also improved the overall monitoring process. Additional improvements such as online data gathering (every 5

days), monthly report submission of USG cases and abortions, USG device inspections are mandatory to monitor facilities across the country.

Figure 28: Timeline of developments of PNDT 1994 and PCPNDT 2003 legislations in India



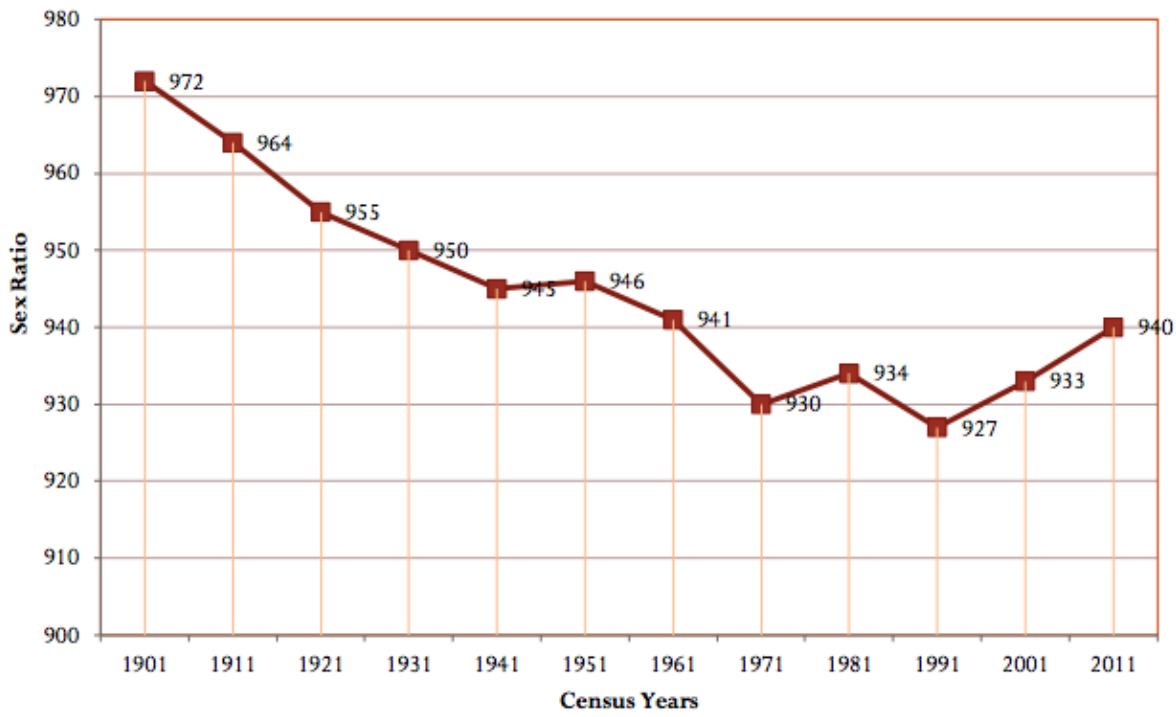
5.5 Current evidences and study rationale

5.5.1 Census data

Census data is a commonly used resource in India to monitor the national sex ratio. This follows the population records, which is updated every ten years at national level. The record contains the population from all age groups, which is used to calculate number of females per 1000 males. This approach is in practice in India to monitor population demographics. The census record of the government calculated the ratio between 1901 and 2011. In 1901 the ratio was 972 (972 females per 1000 males), which showed reduction over a time period to 940 in 2011 (940 females per 1000 males) [138]. The recent three census records (1981 to 2011) are important to consider due to the introduction of ultrasonography devices in the same time period as explained earlier. The ratio was 934 in 1981, which was reduced to 927 in the 1991. The ratio showed an increase to 933 in 2001 and 940 in 2011 (Figure 29).

At global level sex ratio is calculated by dividing total number of males by the total number of females. For example, the data in 1901 from India outlined above could also be expressed as follows: 972 females per 1000 males, means the sex ratio is 1.02 (1000/972= 1.02).

Figure 29: India census data from 1901 to 2011



Source: Register General, Census of India, Government of India 2011.

Note: Number of females per 1000 males are mentioned on the Y-axis.

An important limitation of the data outlined above is inclusion of population of all age groups, which does not represent the ratio at birth The government addressed this by analysing sex ratio of children less than 6 years old. Though the census data reported an increase in number of females from in 2001 and 2011 (compared to 1991), the national data limiting to children (0-6 years) showed decrease (Table 21) [138]. The data presented below revealed the rapid decline in number of female births between 1981 and 2011 at national level in the time period when the ultrasonography technology was commercialised in Indian healthcare system.

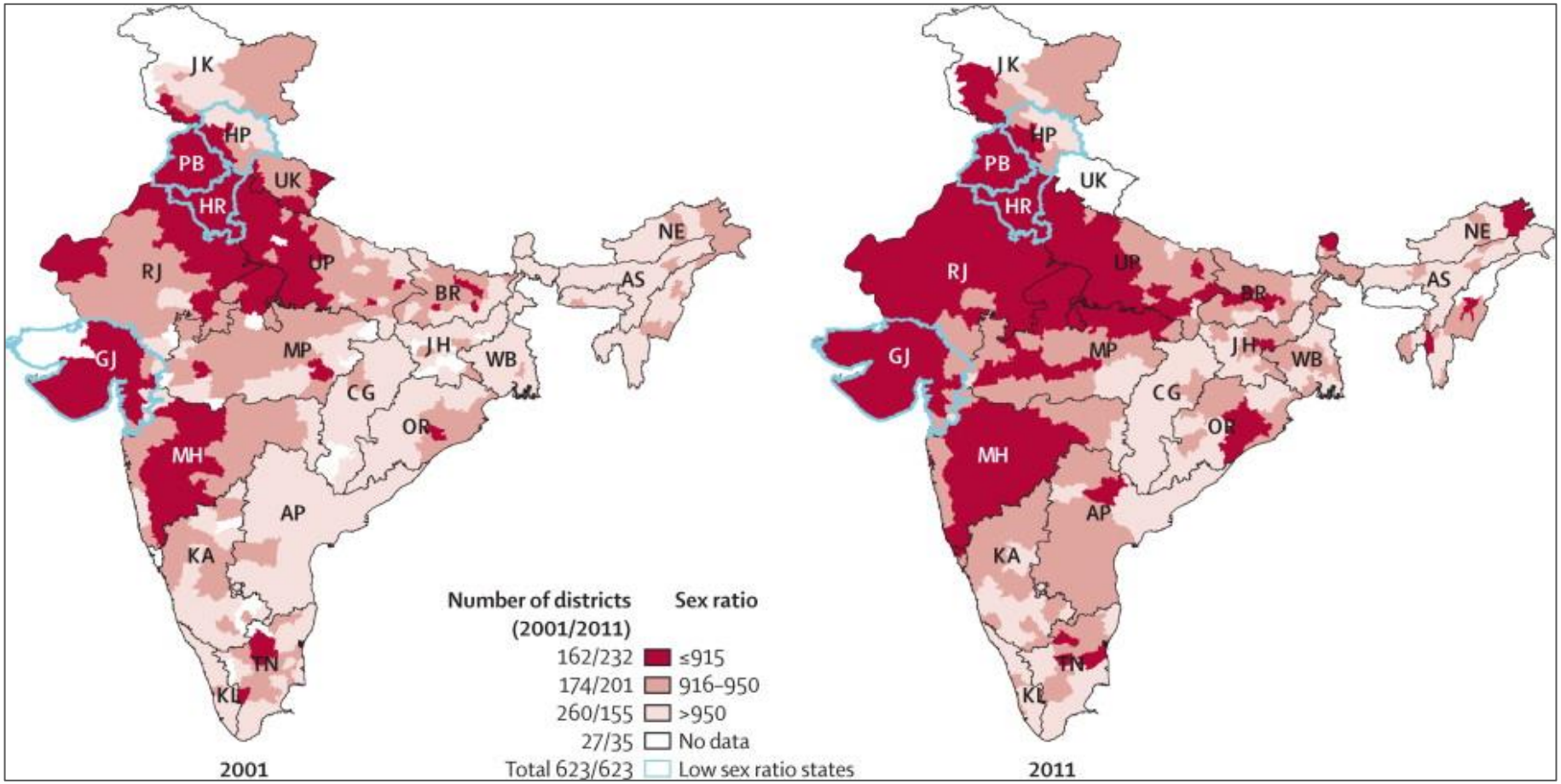
Table 21: Sex ratio among 0-6 year old children (generated using national data)

Year	Number of girls (0-6 years old) per 1000 boys (0-6 year old)	Sex ratio
1961	978	1.02
1971	965	1.03
1981	964	1.03
1991	945	1.05
2001	927	1.07
2011	914	1.09

A graphical representation by Jha et al showed the change in the ratio in 2011 compared to 2001 [22] (Figure 30). Data were useful in understanding the effect of USG technology, however this is likely to be influenced due to the overall neglect associated with a girl child compared to a male offspring. One of the reasons is the existence of a gender-gap due to cultural importance given to men in Indian society as outlined previously. A qualitative analysis in Delhi showed that women perceived male birth as a precious child compared to female [142].

This further showed data gaps, and created opportunity to analyse sex ratio at birth, which is a true measure of change in number of female births over a time period. The ratio at birth is less likely to be affected by issues (related with gender-gap), child care differences (within male and female child), and may provide detail investigation of the impact of USG access on female births.

Figure 30: Male to female ratio in 2001 and 2011 based on national census data in India



Source: The figure is obtained from a publication of Jha P et al [22]

5.5.2 Current evidences and study rationale

Currently published data from India is widely based on census data, where population across all age group were analysed. Published evidences also discussed on societal issues related with the female foeticide. There is limited research on sex ratio at birth, which has potential to inform effects of ultrasonography devices on male:female ratio.

A recent study by Sahni et al using 100 years of hospital data reported that sex ratio at birth declined significantly after 1980 [20]. However, this analysis used data from only one hospital in Delhi (total 321,911 births over 11 decades). Other two analyses by Jha et al [22,151] utilised national survey data includes Special Fertility and Mortality Survey (SFMS), and census data from 1991 to 2011. The SFMS had limited samples analysed at national level (total 133, 738 births). Findings from the census data used sex ratios by birth order.

To our knowledge, no study was published where temporal change in sex ratio at birth was analysed over a time period where annual births were analysed. The birth registration was initiated in India after the introduction of the 'Birth and Death Registration Act 1969 [152]. The act is applicable across the country where Indian state and territories are required to record number of births along with the location, and sex of a new-born. This data is collected manually in both rural and urban regions on regular basis. Such longitudinal data provides opportunity to investigate sex ratios at birth to examine trends of both male and female births following the introduction of ultrasonography devices, and also the legislation in India to prevent female foeticide.

Therefore, we conducted the study using the Maharashtra state birth registry data, collected for a period of 32 years (1980 to 2011).

5.6 Study objective and data source

5.6.1 Study objective

To investigate trends of male:female sex ratio at birth, of Maharashtra state of India, over 32 years of period (1980 to 2011).

As this period spans both the widespread introduction of ultrasound scanning in India, and also the subsequent legislation banning its use for sex-determination of foetuses, we were able to explore the hypothesis that any trends observed might be a consequence of these events.

5.6.2 Maharashtra State Birth Registry

Births are recorded on a regular basis to maintain the state and national population data in India. Each state collects and stores information from a specified geographic region allocated by the national government. In Maharashtra state, such records are maintained at the Civil Registration Department of Maharashtra Government based in Pune city.

The Civil Registration System holds information on births in the state that includes sex and birth location (defined as urban or rural). The registration department also supports and monitors data collection procedures across the state. The process of birth registration is explained below (Figure 31).

I. Village or urban community level process

Healthcare workers at village/urban community level collect information on newly identified pregnancies and also on delivery outcomes. The process is uniform in Maharashtra, where trained healthcare staff; commonly antenatal midwives collect information on each birth, which is recorded in the population catalogue. Each nurse has responsibility for 8 to 10 villages. In urban areas, one nurse caters for a population of 10,000 within a fixed urban area for data collection. Midwives also receive support from local healthcare staff involves male healthcare worker, ASHA or USHA workers (Accredited Social Health Attendant/Urban Social Health Attendant), who assist in conducting hospital and house surveys to record new births. Data collection on births is conducted on a monthly basis primarily by a midwife, which is handed to the block level government health office.

II. Block level records

Block level health office summarises data from all villages under their territory, and submits data to the district health office every month. Number of villages in each block varies; for example, Tuljapur block of Osmanabad district has 107 villages, while Lohara has 45 villages. Each block is responsible for birth registration from their allocated villages/communities.

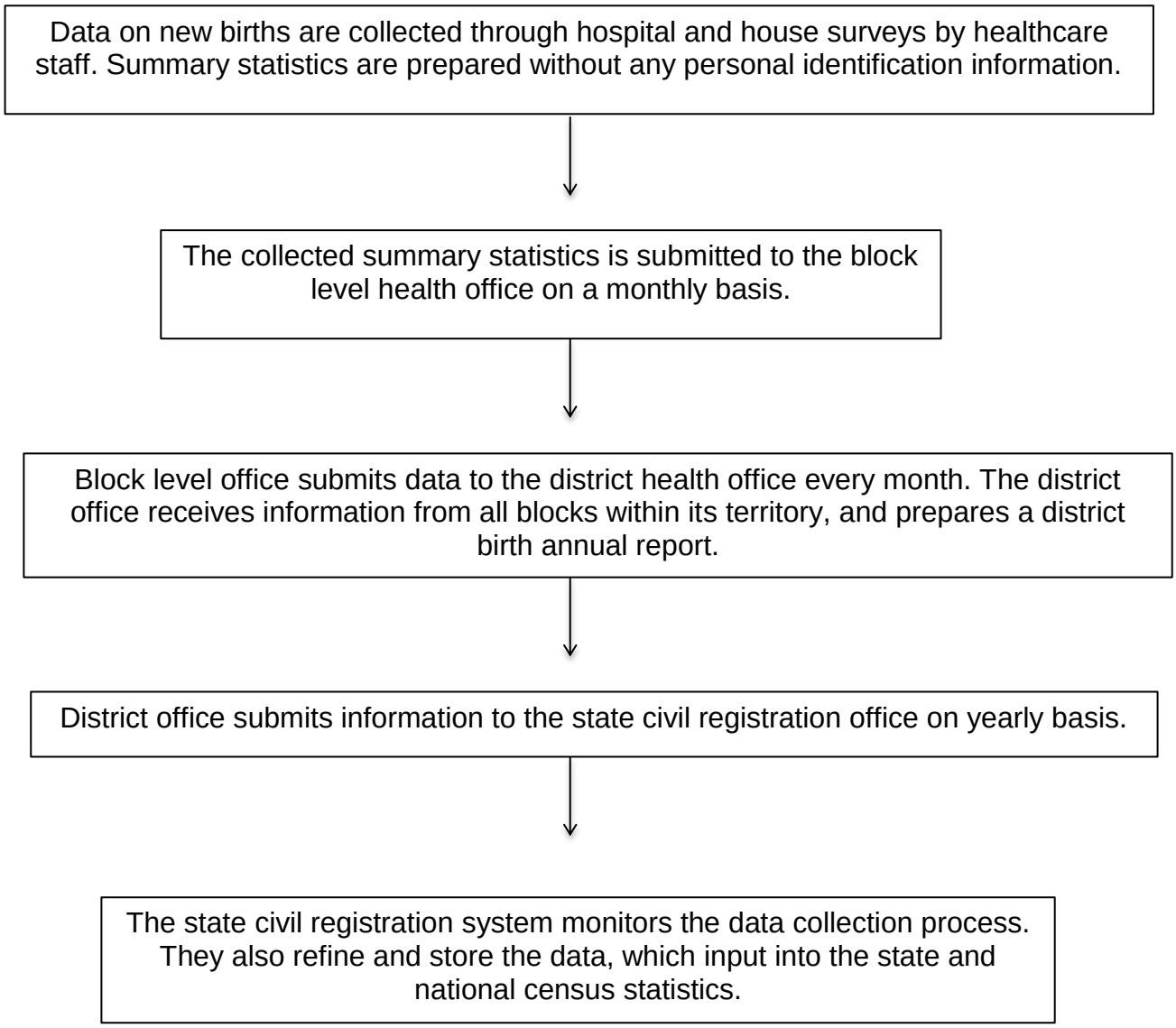
III. District level records

District health office monitors the data across allocated blocks, and submits record to the State Civil Registration office (located in Pune city of Maharashtra) on yearly basis. Number of blocks in district varies; for example, Osmanabad district where MAS study was conducted has 8 blocks having 741 villages in total.

The three-tier system (village/urban community, block, district) is well established for such information collection, and is also used for state and national health programmes implementation and monitoring.

The primary benefit of early birth registration is linked with the Public Distribution System (PDS) of food items [153]. The PDS system provides resources (such as wheat, rice, sugar) on a monthly basis at subsidised rates. The PDS card is allocated for each family where total family members are registered. The amount of food issued depends on the number of family members, thus early birth registration increases the food allowance. Any addition of new family member on the PDS card demands birth registration certificate, which is provided by local government office/nurse, thus birth registration generally has high response rate.

Figure 31: Overview of birth data collection process implemented in Maharashtra State of India (information flow from top to bottom)



5.7 Methods

5.7.1 Study design

The study was a longitudinal analysis of all live births registered from 1st January 1980 to 31st December 2011 with the Maharashtra State Government Civil Registration office. The summary statistics for the outlined 32 years of period were provided by the registration office.

5.7.2 Data collection and quality

The PI (PhD candidate) approached the director of the Civil Registration Office located in Pune city of Maharashtra. The director provided 20 minutes of appointment where the PI discussed on research plans and proposed outcomes. On receiving permission from the director, the PI (PhD candidate) worked with the statistician and a data storage officer, who provided data sheets for 32 years period. The information was recorded manually as it was not available in electronic format. The data were obtained from the original annual reports prepared by the registration office over the study period. This data extraction was performed by the PI at the State Civil Registration Office, Pune. A data assistant (employed within the MAS) then entered the data in computer. The PI ensured that all electronic entries made by the data assistant match with the original dataset. The register office was approached only once, and the data collection and verification was completed within 30 days.

The state civil registration department collects and stores data from across Maharashtra state, and the process is refined periodically to improve the accuracy. The registration process outlined earlier (Figure 31) is in practice since 1969 (Birth and Death Registration Act 1969). This is also used for the national census statistics. Qualified nurses collect

information with support from local healthcare staff. The established support system ensures that all births are recorded within the allocated geographic region. Statisticians based at the registration office verify data from all districts on annual basis. The certified summary statistics are published in the form of annual reports. This verified record of 32 years were used in the birth weight analysis.

5.7.3 Statistical analysis

The electronic data had total number of male and female births over 32-year period (1980 to 2011) along with location of birth (rural/urban). The following formula was used to calculate sex ratio at birth for each year.

$$\text{Annual sex ratio at birth} = \frac{\text{Total number of male births in the calendar year}}{\text{Total number of female births in the same calendar year}}$$

Increase in the sex ratio indicates decrease of the denominator, meaning decreasing in the number of annual female births. Similarly, decrease in the sex ratio indicates increase in female births or decrease in male births.

The calculated sex ratio at birth of Maharashtra state for each year was entered in computer system (in total 32 ratios for 32 years). For the stratified analysis of rural and urban areas, sex ratios were calculated using the outlined formulae and the same were recorded in computer.

Joinpoint regression analysis software was used to assess changes in linear trends over the time period (1980-2011). This regression model allows identification of the time of significant changes in linear trend slopes. The Joinpoint regression software was obtained

from the National Cancer Institute, United States of America [154]. The software is available for research and education purposes in the public domain. Additional information on the software programme and analysis methods is outlined in appendix 11.

The subgroup analyses stratifying by urban or rural location of birth were also performed in an identical manner. To assess if there were any statistically significant differences between the trends in the sex ratio over time between urban and rural areas, the annual difference was calculated for each year and then plotted against time for both groups labelling the baseline year as zero to allow interpretation of the intercept. This analysis used Stata Statistical Software (v13.1, Texas, USA). As each year provided only one data point with no variance, linear models were used for all analyses with no adjustments for autocorrelation. Statistical significance was considered as a p value less than 0.05.

5.8 Results

5.8.1 State level sex ratio assessment

From 1980 to 2011, the total number of live births registered in Maharashtra State increased from 1,250,643 to 1,925,204. The annual sex ratio increased from a baseline of 1.11 in 1980 to a maximum value of 1.23 in both 2003 and 2004, before decreasing to 1.16 in 2011 (Figure 32). There was a significant increase in the annual sex ratio at live birth from 1980 to 2004 of 0.005 units/year ($p<0.001$), and a decrease of 0.009 units/year after 2004 ($p=0.01$).

Figure 32: Temporal trends in sex ratio at live birth for total population of Maharashtra State for 32 years of period (1980 to 2011)

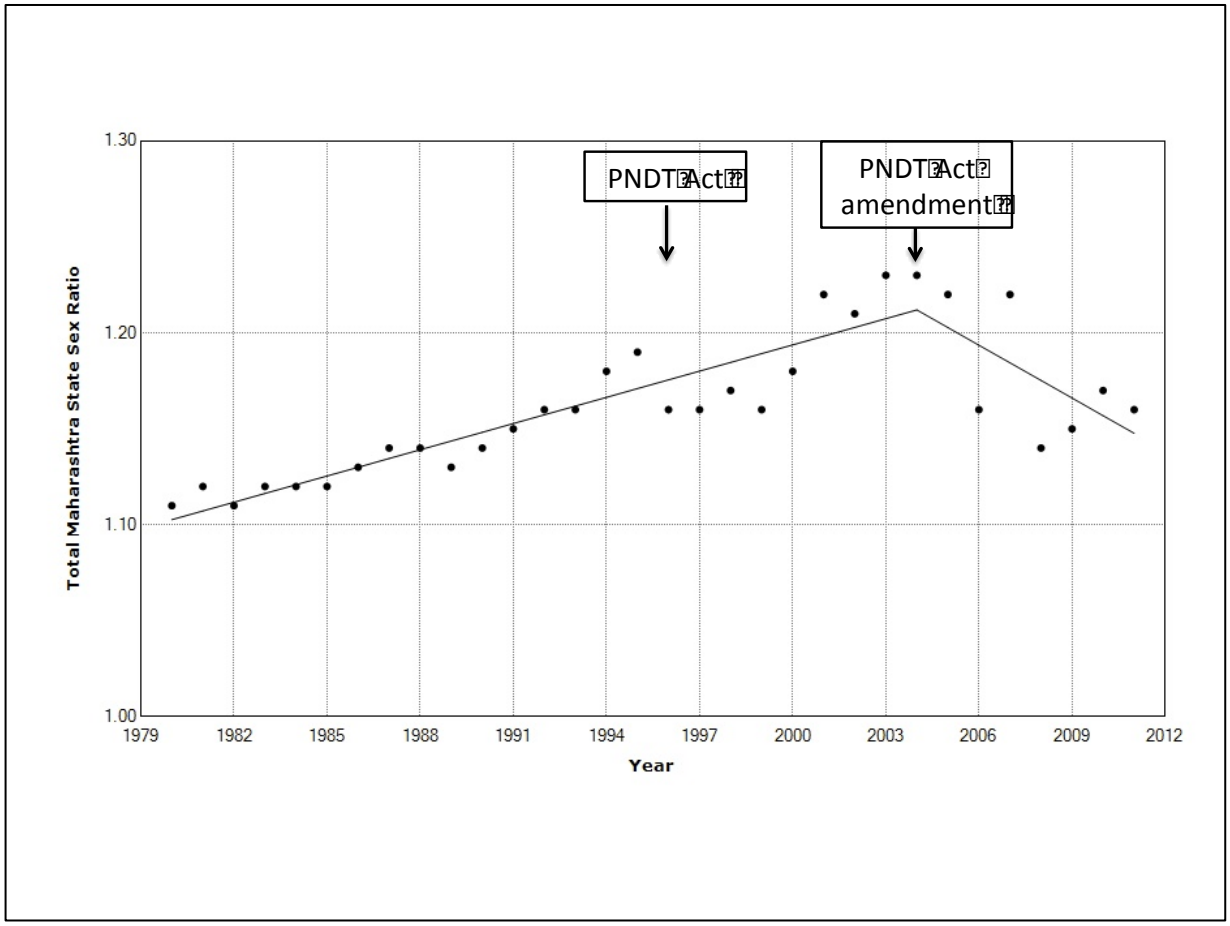


Figure 32 footnotes:

- PNDT Act is Prenatal Diagnostic Techniques Act introduced in the 1994.
- PNDT Act amendment is also known as PCPNDT 2003.

5.8.2 Stratified analysis using urban and rural births

In the urban population, the annual sex ratio increased from 1.08 in 1980 to a maximum value of 1.23 in 2005, before decreasing to 1.14 in 2011 (Figure 33). There was a significant increase in the annual sex ratio at live birth from 1980 to 2006 of 0.005 units/year ($p<0.001$) and a decrease of 0.014 units/year after 2006 ($p=0.02$).

In the rural population, the annual sex ratio increased from a baseline of 1.13 in 1980 to a maximum value of 1.32 in 2004, before decreasing to 1.19 in 2011 (Figure 33). There was a significant increase in the annual sex ratio at live birth from 1980 to 2003 of 0.005 units/year ($p<0.001$), with a decrease of 0.009 units/year after 2003 ($p=0.08$).

For most years the sex ratio was higher for births in the rural region than the urban population. In the regression analysis of the difference in sex ratio between urban and rural location, there was a higher intercept at the baseline year for the rural sex ratio compared to the urban data which was significant (+0.05, 95% confidence intervals +0.03 to +0.08), however there was no significant difference between the regression coefficients for sex ratio over time ($p = 0.38$).

Figure 33: Temporal trends in sex ratio at live birth for population of Maharashtra State stratified by urban and rural location for 32 years of period (1980 to 2011)

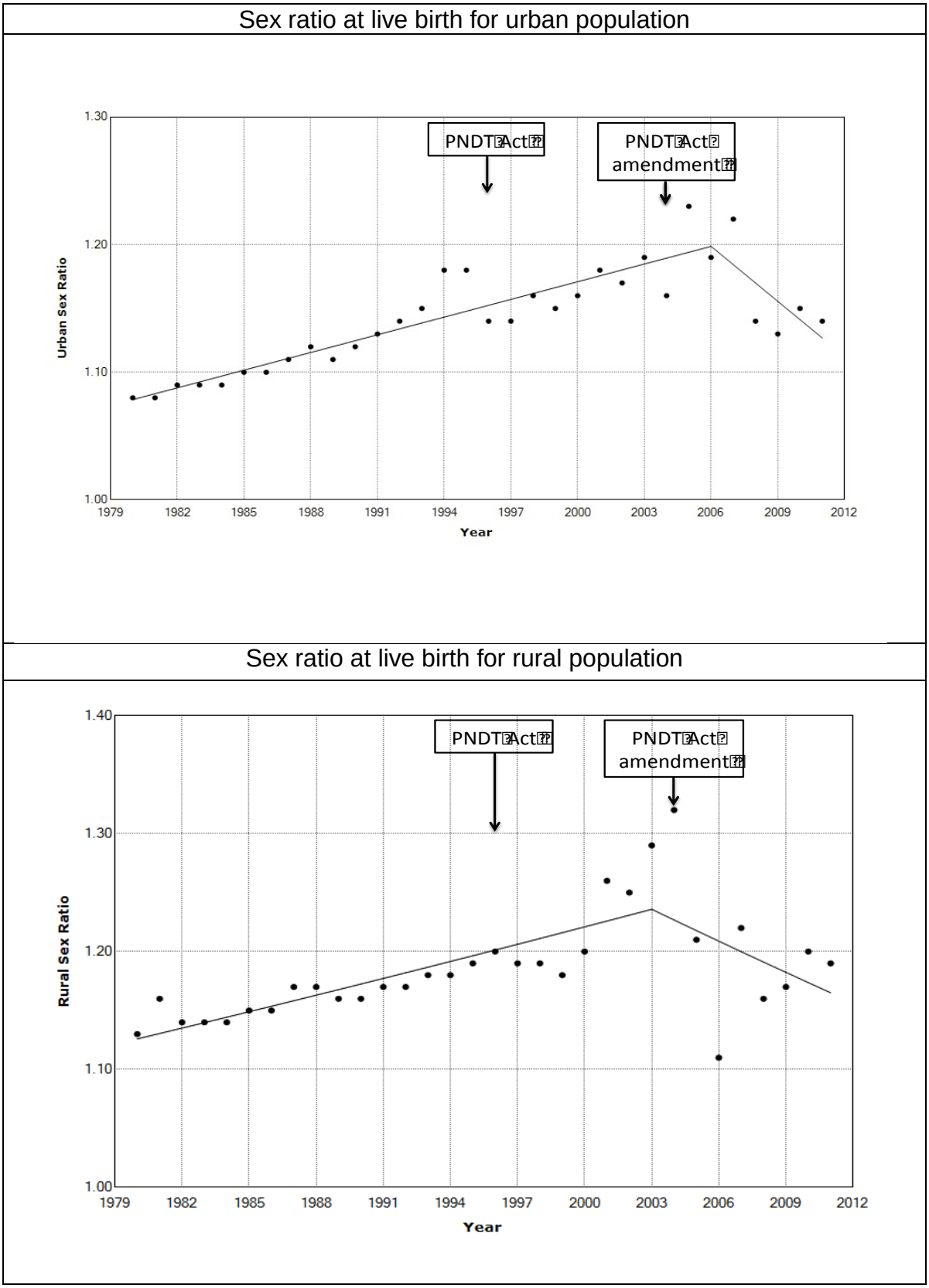


Figure 33 footnotes:

- PNDT Act is Prenatal Diagnostic Techniques Act introduced in the 1994.
- PNDT Act amendment is also known as PCPNDT 2003.

5.9 Discussion

5.9.1 Principal findings

This is the first longitudinal analysis of temporal trends in sex ratio at live birth in Maharashtra State, which spans a period of time when ultrasound scans became widely available, and subsequent legislation banned its use in pre-natal sex determination in India. Our data demonstrate a clear increase in the sex ratio at birth that peaked in 2003 and 2004 and then decreased in subsequent years after the introduction of PNDT amendment (PCPNDT 2003). Sex ratios at live birth were consistently higher in rural locations compared to urban.

5.9.2 Strengths and limitations

The strengths of our data are the availability of the number and sex of live births over a period of 32 consecutive years from a well-defined population living in Maharashtra State. To our knowledge this is the first analysis from India, where longitudinal data from government birth registry were used to investigate impacts of ultrasonography legislation on female births. While visual inspection of these data suggests temporal trends, the use of hypothesis free Joinpoint analysis permits optimal fitting of these data, and most importantly the identification of periods of time when the linear trends change. These can then be compared to the timing of known societal interventions such as the introduction of laws banning pre-natal sex determination to assist interpretation of these observations. This data driven approach to analysis was preferred to other statistical approaches such as interrupted time-series analysis, as these depend on a prior hypothesis supported by sensitivity analyses, and the multiple comparisons generated can complicate interpretation of any associations

observed. We have also had the opportunity to explore trends in sex ratio at live birth stratified by urban or rural location, where differential access to ultrasound scanning and cultural practices may vary.

It is important to consider that our analysis has a number of limitations. These are explained in turn below:

- Generalisability
- Limitations related with the dataset
- Confounders

Generalisability: The study observations are applicable to Maharashtra State, and not necessarily generalisable to other states in India, as there are large differences in rates of sex-specific foeticide across the country due to cultural differences in attitudes towards females across India. Our analysis demonstrates clear temporal associations between annual sex ratios at live birth, and legislative changes in 2003, but cannot prove causality. However, they are the best data available on sex-specific foeticide in Maharashtra State, as these data cover a large population across the state over 32 years of period.

Limitations related with the dataset: To our knowledge, there was no verification of collected birth data/entries over the study period. Birth registration is a continuous and complex process, which involves a large number of healthcare workers across the state and some degree of reporting error may have occurred. However, it is not possible at this stage to retrospectively verify the accuracy of each case (birth) recorded in the registry, which is a major limitation.

Confounders: Further, the study was only able to assess overall trends in the sex ratio with no information on maternal factors such as education, socioeconomic status, gravidity, number of living female and male children, total children, history of abortion, which may have provided insights into factors associated with sex ratio trend and preference for male offspring.

5.9.3 Comparison with current evidence

Our analysis of the total population demonstrates a significant increase in the sex ratio at live birth from 1980 to 2003-2004, and then a subsequent decrease in sex ratio. We consider that the increase in the sex ratio is consistent with the hypothesis of both increasing availability and acceptability of ultrasound scanning during this period, resulting in foeticide of females *in utero*. The reasons for the decrease in sex ratio after 2004 may be a consequence of the legislation banning sex specific foeticide that was strengthened in 2003 finally having an impact on access to pre-natal sex determination. These changes included regulating the sale and use of ultrasound diagnostic technology along with formal oversight by regulatory bodies who have the authority to prosecute offenders, at both institutional and individual levels [155]. Alternatively, a combination of adverse media publicity and societal education about the practice of sex specific foeticide may have changed attitudes to the preference for male offspring or the acceptability of this practice [150]. It is disappointing that the initial introduction of legislation in 1996 had no impact on the increasing trend of male:female sex ratio at live birth, and these data suggest that future legislation in this sensitive societal area needs to be accompanied by the threat of tangible punitive sanctions for offenders.

To our knowledge there are no comparable population-based studies of temporal trends in sex ratio in India over the past four decades. One study of hospital deliveries in New Delhi presented data on the sex ratio at live birth for the middle year of each decade from 1900, and reported that this increased from 1.06 in 1985 to 1.16 in 2005 [20], although the authors note that these data are not representative of local state and Indian society as over 60% of deliveries take place outside medical institutions. However our dataset is provided by the government, which included urban and rural regions across the state, where both hospital and home deliveries were recorded.

5.9.4 Conclusion

We report that the sex ratio at live birth in Maharashtra State increased substantially from 1980 to 2005, and subsequently decreased. The decrease in the sex ratio after 2005 is mainly due to increase in female births, which represents the beginning of a continuing trend that will result in more balanced sex ratios in India in future generations that will be to the benefit of society. Importantly, the increase in female births is a result of PNDT amendment (PCPNDT 2003), which developed a team in each district to ensure strict implementation of the legislation in Maharashtra state to prevent misuse of ultrasonography devices. In conclusion, our analysis shows that appropriate implementation of the legislation is useful in improving the Indian sex ratio.

Chapter 6. Conclusions and recommendations

6.1 MAS objectives and chapter overview

Maharashtra Anaemia Study had three main sections (adolescent girls, pregnant women and Maharashtra birth registry data). To recap, MAS objectives were as follows:

1. To estimate anaemia prevalence, and identify individual and village level risk factors associated with anaemia among adolescent girls (13 to 17 years) in Maharashtra. The secondary objective was to conduct a comparison of the non-invasive haemoglobin technology (NBM 200) with Sahli's hemometer in this adolescent girl population.
2. To estimate anaemia prevalence, and identify individual and village level risk factors associated with anaemia among pregnant women in Maharashtra. The study also aimed to assess risk factors associated with low birth weight. The secondary objective was to validate the non-invasive haemoglobin technology of (NBM 200) using an automated cell counter (Sysmex XP-100) in this rural pregnant women population.
3. To study temporal changes in the sex ratio at birth of Maharashtra state over a period of 32 years (1980 to 2011) using anonymised routinely collected healthcare data to investigate a potential effect of ultrasonography legislations.

The important findings on each objective are summarised in section 6.2. Information on clinical and public health implications are mentioned in section 6.3. The last part of the thesis provides scope for future research.

6.2 Conclusions

6.2.1 Prevalence and risk factors of anaemia in adolescent girls in Maharashtra

Anaemia prevalence was 87% (of 1010) among adolescent girls with majority of participants had moderate and mild anaemia. Anaemia likelihood increased significantly with age (OR 1.41 per year, 95% CI: 1.17 to 1.70). Factors associated with decreased anaemia risk in the fully adjusted model were age, mid upper arm circumference (MUAC) ≥22 cm (OR 0.51, 95% CI: 0.31 to 0.82) and ≥3 days/week consumption of fruit (OR 0.35, 95% CI: 0.23 to 0.54). Village factor such as piped water supply was associated with higher Hb levels (linear regression β: 0.61, 95% CI: 0.39 to 0.82, p<0.001). In the fully adjusted logistic regression, piped water supplies and its regularity (p<0.001), toilet facilities (p<0.001), and access to government nurse (p=0.002) associated with lower anaemia. Results from the NBM 200 reported a wide agreement levels in the Bland-Altman analysis (mean difference of -2.70 g/dl, 95% CI: -2.84 to -2.55) demonstrating an overestimation of Hb by the NBM 200 compared to hemometer. The NBM 200 showed low sensitivity (23.6%) and moderate specificity (61.8%) for the diagnosis of anaemia.

6.2.2 Prevalence and risk factors of anaemia during pregnancy and low birth weight in Maharashtra

Findings from 287 pregnant women from 3 to 5 months of pregnancy showed high anaemia prevalence (77%). The mean corpuscular volume (MCV) of 80.5 fl/cell, (SD: 7.22, range: 53.4 to 93.8) was less compared to the first and second trimesters thresholds. Increased risk of anaemia was seen in women with consanguineous marriages (OR 2.41, 95% CI: 1.16 to 5.01) after adjustment for potential confounding factors. Post-delivery data from full-term singleton live births showed the prevalence

of LBW babies was 7%. Consanguineous marriage was a major risk of LBW babies in our study population (OR 5.68, 95% CI: 1.58 to 20.32). Village risk factors showed lower likelihood of maternal anaemia with regular access to government nurses. However none reported significant association in the adjusted analysis. The NBM 200 validation showed overestimation of Hb levels and underestimation of anaemia similar to the adolescent girl study. Bland-Altman analysis showed a mean difference of -1.8 g/dl (95% CI: -2.06 to -1.71) indicating a systematic overestimation of Hb level by the NBM 200. The device showed low sensitivity (33.7%; 95% CI: 27.3 - 40.5) but high specificity (91.8%; 95% CI: 81.9 - 97.3) for the diagnosis of anaemia.

6.2.3 Impact of Ultrasonography legislation on births in Maharashtra

The annual state sex ratio increased from a baseline of 1.11 in 1980 to a maximum value of 1.23 in 2003, before decreasing to 1.16 in 2011. We noted an increase in the annual sex ratio at live birth from 1980 to 2004 of 0.005 units per year ($p < 0.001$), and a decrease of 0.009 units per year after 2004 ($p < 0.01$). The 32 years longitudinal data showed a significant increase in the sex ratio at live birth from 1980 to 2004, and then a subsequent decrease in sex ratio. We consider that the increase in the sex ratio is consistent with the hypothesis of both increasing availability and acceptability of ultrasound scanning during this period, resulting in foeticide of females in utero. The reason for the decrease in sex ratio after 2004 is due to the strengthening of the legislation banning sex-specific foeticide. In summary, the sex ratio at live birth in Maharashtra state increased substantially from 1980 to 2004 and subsequently decreased following the revised legislation.

6.3 MAS Outcomes

Our research demonstrated that anaemia prevalence is very high in both adolescent girls and pregnant women suggesting an urgent need to address this important issue at wider level in Maharashtra state, and also in India.

The recent Indian National Family Health Survey has recognised anaemia in the top 10 public health problems in the county. However, there are many areas such as Marathwada region, where data on anaemia are limited.

It is important to note that anaemia is not limited to girls and pregnant women, but also observed in boys, men, and elderly people, thus it should be considered comprehensively while planning interventions at both community and individual level.

Based on MAS findings, we have provided recommendations to address high prevalence of anaemia. Additional suggestions are also made to address female foeticide problem, which is an important health issue in the country that not only affects women's immediate health but also the whole population considering the subsequent effects on pregnancy and population dynamics.

The following recommendations are delineated in the three sections.

- Recommendations for public health practitioners
- Recommendations for healthcare providers
- Recommendation for future research

6.3.1 Recommendations for public health practitioners

A key finding of this research is extremely high prevalence of anaemia in both adolescent girls (87%), and pregnant women (77%). Although we identified some risk factors, other traditional influences such as dietary components did not report any strong association. We have outlined below key areas for public health practitioners to address the issue.

- It is important to develop comprehensive strategies to tackle current health and infrastructural challenges in rural settings. This includes improving anaemia detection and treatment services through government health centres. Training healthcare providers is an important step involved in the strategic planning.

- Scaling up community education and ensuring it reaches all individuals is vital to improve knowledge and awareness about anaemia particularly in rural areas. This could be provided through school platform, or through village health sanitation committees, which will allow integrating anaemia and health education at community level.

- Community resources such as water supplies, sanitation facilities and access to hospitals were associated with higher haemoglobin levels. This emphasises outlined suggestions to strengthen health and social systems. Anaemia being an important health issue needs constant surveillance, financial support and advocacy from the government. Therefore, NGOs such as HMF will have an opportunity to establish platform at state and national

level in collaboration with agencies such as National Institute of Nutrition, and other government departments in India.

- Data from the MAS project has opened range of opportunities, not limited to research, but also to plan health programmes to improve overall community health. This will be useful to monitor community health structure, access, and services to address high anaemia prevalence.

- Medical research into causes of anaemia and reasons for such high prevalence in rural population will help in strengthening treatment plans. This will permit to improve anaemia prevention, and health promotion services at both state and national level. Therefore, it is vital to continue research in anaemia covering rural and urban population.

6.3.2 Recommendations for healthcare providers

Private clinicians and other government healthcare staff such as nurses, ASHA workers are major part of health services in rural India. The following suggestions are important to improve anaemia related health services.

- Considering the high prevalence, anaemia screening should be performed on regular basis for population such as women and adolescent girls every six months. The NRHM division of the central government has provided recommendations for the screening, which should be followed by the government authorities as well as private healthcare providers particularly in rural areas where health infrastructure is limited. Collaboration between

government and private healthcare systems will allow expansion of anaemia health services.

- Mid upper arm circumference points to nutritional status and showed association with anaemia in our research. In areas where health facilities are limited, such clinical parameters could be easily assessed, which will facilitate malnutrition and also anaemia identification during clinical visits. This will improve identification, treatment and referrals for existing cases.

- Anaemia risk in adolescent females increases with age; hence early diagnosis could be reached by focusing on older girls, which will help to target interventions. This may help to reduce prevalence, and will ensure appropriate utilisation of limited healthcare budget. Simultaneously, younger girls could be involved in anaemia prevention programmes.

- Dietary items such as fruits are positively associated with Hb levels. As eating fruit is well recognised as part of a healthy diet, clinicians and government healthcare staff can provide nutritional and dietary advice particularly in rural areas where other medical expertise are not accessible.

- It is also important to understand diets of young girls in rural Indian society, where food preference/choices are often given to men and boys compared to females. Such initiatives could be integrated within the government nutrition programmes, which will increase utilisation and will provide benefits to wider population groups.

- Consanguineous marriage was found to be a modifiable risk factor for maternal anaemia and low birth weight. Clinicians being the first point of contact are in position to shoulder counselling and referrals to prevent adverse impact on child health. Health education is an important area to address this as outlined earlier. This could be also integrated with village education committees, which are currently functional in rural India.

- Clinicians can play an important role in delivering health education in hospital setting, which can benefit to reduce anaemia prevalence at wider level. However such programmes should provide necessary training in nutrition to clinicians to ensure quality and consistency of interventions.

- Both government and private healthcare providers are major players in providing family education to prevent female foeticide. Grass root healthcare workers from both government and non-government agencies can form a foundation to provide first-hand information on female foeticide, and its impact on women health and also on society. This will provide opportunities to initiate community-based programmes to achieve a more balanced sex ratio in the Indian communities.

6.3.3 Recommendation for future research

Based on findings from Maharashtra Anaemia Study, the following research programmes are important to address the high prevalence of anaemia.

- Conduct studies on anaemia using venous blood samples primarily in adolescent girls as currently no such studies are available from Marathwada

region of Maharashtra. This is important to further elucidate the potential causes/types of anaemia which are still largely uncertain in this population and other population in India. There is an urgent need to conduct advanced medical assessments particularly involving young girls to study blood parameters such as vitamin B12, folic acid and serum iron levels that may provide additional insights into the causes of anaemia. Better understanding of such risks will help in designing interventions to reduce anaemia at young age. Future interventions will need to be precise to address exact nutrition deficiency, and the same should be considered while planning preventative strategies.

- Conduct studies focusing on benefits of locally available food items to reduce anaemia. This includes fruits and other accessible resources with potential to improve haemoglobin levels.

- Assess availability and supplies of medical supplements provided by the government to reduce anaemia. We observed that only 2 of 37 schools in the area (where the study participants were enrolled) received iron supplements from the district health department, with the majority being unable to provide medicines due to lack of regulated supplies.

- More Investigation on the response to currently advised anaemia treatment would help in strengthening interventions. This will provide data on treatment access, adherence, and also attitudes of beneficiaries towards anaemia treatment.

- There is an opportunity to investigate the response to current treatment plans and challenges in uptake of anaemia treatment in both adolescent girls and pregnant women.

- Plan antenatal studies since consanguineous marriage is common in Indian culture, and is identified as a potential modifiable risk factor. Such studies will improve understanding on the role of consanguineous relationship with maternal anaemia and pregnancy outcomes.

- Research studies on intrauterine growth, malnutrition and anaemia are important to understand the chronic health problem. This may help to target interventions, and may be useful in planning prevention programmes in the near future.

- Along with the quantitative studies, qualitative studies to assess anaemia knowledge, health priorities should be evaluated. This will help in planning public health and education programmes for rural population.

- Limited information is currently published on adolescent boys from Maharashtra state, which requires an independent inquiry to understand anaemia more widely in this context.

- Research to improve NBM 200 technology is necessary. To our knowledge, MAS is the first study, where the non-invasive technology is evaluated in a real world setting, providing opportunities to test the device in an area where high anaemia prevalence is documented. The portable device has the ability to revolutionise anaemia screening; however, the technology needs to be improved substantially to provide accurate readings in line with the accepted gold standard, before it can be implemented on a wider level. As most published studies, including our own, suggest a systematic overestimation of Hb values by the NBM 200. It may be possible for the manufacturer to improve the performance by recalibrating the sensor or algorithms that process the data collected for population with various skin types. For example, if the melanin content is interfering with Hb measurements, then this will need further attention. In future field-based research in developing countries following improvements to the sensor is required, before advocating the adoption of non-invasive technology for haemoglobin measurement and anaemia diagnosis.

- Monitor ultrasonography facilities and sex ratio at birth to identify and reduce female foeticides incidence. Maharashtra birth registry study is the first showing possible method to monitor male and female births, which was not been explored elsewhere in the country. The persistent monitoring against timed interventions will hopefully result in more balanced sex ratios in India benefiting young girls, women and society at large.

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8. Appendices

Appendix 1: Study village list

Total villages: 35
Population: 65,421

For adolescent girl data collection the first 34 villages were approached with the objective to recruit the first 1000 adolescent girls (villages 1 to 34 outlined in the list below were included in the adolescent girl study).

Participation was open for all eligible pregnant women from 35 villages. No pregnant woman was identified from the village 'Aarni' (No 11) during the recruitment period (24th April 2014 to 31st December 2015), thus recruited participants were from the remaining 34 villages.

No	Village	Village population	Total number of eligible adolescent girls (13 to 17 years)
1	Nagral	750	17
2	Rudrawadi	773	27
3	Hipparaga Taad	900	30
4	Gulhali	932	19
5	Bendkal	940	31
6	Dhangarwadi	1000	32
7	Lohara Khurd	1051	43
8	Dahitana	1059	28
9	Wadgaon Wadi	1073	31
10	Wagdhari	1120	34
11	Aarni	1232	27
12	Sarati	1290	21
13	Harali	1295	42
14	Shirgapur	1300	32
15	Sayeed Hipparaga	1357	37
16	Bijanwadi	1370	55
17	Fulwadi	1416	56
18	Chincholi	1430	53
19	Kasti Khurd	1450	38
20	Basavantwadi	1500	40
21	Mardi	1520	38
22	Nilegaon	2075	77
23	Wadgaon Ganja	2323	83
24	Itkal	2350	55
25	Murta	2500	54
26	Khed	2511	112

27	Nagur	2527	36
28	Bhatgali	2596	56
29	Horti	2651	143
30	Rava Hipparga	2700	85
31	Yevti	2768	100
32	Chivari	3014	93
33	Kanegaon	4048	98
34	Dhanuri	4100	61
35	Khudawadi	4500	114

Appendix 2: Field area photos

(verbal consent obtained, wherever applicable)

1) Road infrastructure: A photograph obtained while travelling to Lohara block. Other than the national highway all internal roads were single carriageway and semi paved (maximum vehicle speed 20 miles per hour).



2) Mobility: HMF provided a vehicle during the study period. All necessary material was taken for each visit. A total of five members travelled for the data collection visit (investigator, assistant, nurse, technician and a driver).



3) Data collection site set up: Data were collected at government health centre/local school/nutrition centre/participant's house. Data collection site was prepared, as shown below (seating mats, table, chair).



4) Health report card: Adolescent girls received reports immediately after haemoglobin testing and participants were interested in reading the details.



5) Temperature: Marathwada experiences very high temperature from February to June each year. In April-May each year the temperature increases to 45 Degree Celsius (a screen shot from the first week of March 2015).



6) Water scarcity: Drought is serious issue in the area mainly due to limited rainfall and absence of reserved forest zones. The main impact is on electricity generation and industrialisation; thus the per capita income is less than half of that in western Maharashtra. This photograph shows the long queue to collect water with a young girl keeping watch. Photograph obtained in ‘Bhatagali’.



7) MAS project: The project is dedicated to all young girls and women resident in the study area. The young girl shown in the second photo below needs to walk 1.5 kilometres to collect water everyday. She is nine years old and from the photograph it is possible to see that her overall physical development is less than that expected for a nine-year-old girl.





Appendix 3: PhD project research proposal

Index

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A) Research title

Maharashtra Anaemia Study: An investigation of factors associated with adolescent health and pregnancy-related outcomes in women from Maharashtra state, India

B) Team

Organisations involved
1) Halo Medical Foundation, India
2) Division of Epidemiology and Public Health, University of Nottingham, United Kingdom

PhD student (primary investigator)
Dr Anand Ahankari (noted as AA/primary investigator in the protocol)

PhD Supervisors based at University of Nottingham, UK

- Dr Andrew Fogarty
- Dr Puja Myles
- Dr Laila Tata

Additional co-investigator involved: Dr Jagannath Dixit, Government Medical College Aurangabad and Government Medical College Latur, Maharashtra, India.

C) Abstract

The aim of the proposed research is to identify factors associated with, adverse pregnancy-related and reproductive health outcomes among women living in Marathwada, India. The project uses a prospective observational approach to investigate individual and village risk factors for being diagnosed with anaemia in populations of pregnant women and adolescent girls living in 35 to 40 villages in the Marathwada Region of Maharashtra. Additionally, the study will use the birth registry record of Maharashtra state to analyse temporal changes in the state sex ratio at live birth over a period when perinatal sex determination has become available (despite being illegal). The project setting is in an area within which the non-governmental organisation Halo Medical Foundation (HMF) coordinates a number of different health and social development programmes and will thus be integrated in HMF's work. This research protocol will provide the background, rationale, aims, objectives and methodology of the proposed study. The methodology section is focused on project area, outcome measures, data collection and analysis methods. Ethical considerations, research limitations and dissemination plans are noted in the concluding section.

D) Introduction

Anaemia is a global health issue, affecting women throughout their reproductive lifespan, but with particularly poor consequences during adolescence and pregnancy. Iron deficiency is the most common cause of anaemia and often exists with other systemic conditions such as nutritional deficiencies, parasite infection, malaria or haemoglobinopathies (1). India has the world's largest anaemic population affecting nearly 55% of women during pregnancy and 60-70% of adolescent girls, thereby negatively impacting women's health and that of their children (1).

Despite recent economic growth, India has the largest number of people living in poverty in the world with 33% of the population earning less than a 1.25 US$ a day, after adjustment of purchasing power parity (6). The state of Maharashtra in the Western part of India has about 109 million people, many of whom experience a combination of poverty, malnutrition

and limited access to healthcare services adversely impacting maternal and child wellbeing. Although the country's economic capital Mumbai is in Maharashtra, there are still a large number of marginalized communities based in the central and eastern part of the state (2). Marathwada is recognised as a particularly deprived region with more than 70% of the population surviving on rainfall-dependent agriculture (2).

Osmanabad is one of the most deprived districts of Marathwada having limited rainfall and few opportunities for education and income generation activities. Literacy was observed to be 55% among women with 27% child marriages. Maternal health data showed that about 80% of pregnant women delivered under medical supervision and 68% access antenatal care across the pregnancy (3, 4). Nearly 75% of pregnant women were found to be anaemic with nearly 10% being classified as severe (3).

Although being classed as a priority area of research as defined by Millennium Development Goals 4 and 5, there have been very few studies on anaemia or women's health in general in rural Maharashtra. One study from this region reported that smaller stature, younger age at marriage and higher parity was associated with an increased risk of anaemia in women (6). Additionally, a recent case-control study from the same geographic region found that maternal weight less than 45 kg, age less than 21 years and haemoglobin less than 11 g/dl were important risk factors for adverse pregnancy-related outcomes (8). Research in another region has showed that iron supplementation improved both haemoglobin levels and growth among adolescent girls living in India (7), but community implementation of this remains extremely limited. A further problem related to pregnancy in Maharashtra is female foeticide. National data from a special fertility and mortality survey from 1998 estimated that about 10 million foetuses may have been selectively aborted in the preceding two decades potentially accounting for the high male:female sex ratio at birth (9). As a consequence, the government of India has passed legislation banning early diagnostic imaging for ascertaining the gender of foetuses (10). It remains unclear, however, whether this has continued in practice, as there have been limited further studies.

E) Research scope

Halo Medical Foundation (HMF) is a non-government organisation working in Maharashtra since 1993. Much of the work of HMF is in maternal health, village healthcare networks, self-help group, violence prevention, education and women's empowerment. The foundation also works in urban areas of Sholapur district focusing on a gender equity programme and maternal and neonatal health initiative covering a population of about 150,000. Within the Osmanabad district, the foundation selected local women at the village level for training as healthcare workers, resulting in about 35 to 40 healthcare staff in the district. Each village has an individual healthcare worker to support pregnant women and adolescent girls. Along with the focus on maternal health issues, healthcare workers are engaged with local governance and support systems to strengthen the women's network.

The proposed research will be conducted collaboratively by HMF and the University of Nottingham to gain an understanding of health data and maternal health issues within the districts. The project encompasses about 35 to 40 villages, where HMF healthcare workers are actively working on an initiative to reduce anaemia among women during pregnancy and also in adolescent girls. This research important for this rural population of Maharashtra as no previous evidence is available on anaemia in this area.

F) Rationale

- Anaemia among women and young girls is highly prevalent in the Indian population with an increasing prevalence in rural and urban areas (12,13). A report by the World Health Organisation suggested that women in the reproductive age group are at maximum risk of acquiring anaemia (1). Published research involving the Indian population showed evidence of the harmful effects of anaemia during pregnancy on maternal health and birth outcomes (8,12,13); however individual and village level risk factors of acquiring anaemia in the Indian population have not been evaluated. Furthermore, research focusing on anaemia during pregnancy from Marathwada region of Maharashtra is not available as this is a very unique and difficult to reach population. This information is crucial for the development, planning and practical implementation of much needed community health services focussing on women's health and could also raise evidence that will be of use in other regions nationally.

- In addition, this study will look at whether the sex ratio has changed over the 30 years as a potential marker of female foeticide. Female foeticide has been a central focus in the state and is a major maternal health concern, however data about female foeticide are very limited and there are no published data available from Maharashtra state. This research particularly focuses on state sex ratio to study temporal changes in sex ratio over a period of at least 32 years (1980-2011) and a further comparison could be made between rural and urban settings (depending on data availability).

- This research will help to quantify the prevalence of anaemia, identify risk factors and highlight potential gaps in the existing healthcare structure. It would possibly provide opportunities to strengthen healthcare services. Finally, this research will establish baseline epidemiological data, which would provide a future platform to conduct health research in anaemia in communities of Osmanabad district.

G) Aims and objectives

1. To identify individual and village level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 35 to 40 villages of Osmanabad district, Maharashtra using an observational epidemiological survey.

2. To identify impact of maternal anaemia on birth weight using pregnant women data.

3. To compare and evaluate application of non-invasive haemoglobin technology in adolescent girls and pregnant women from rural Indian setting.

4. To study temporal changes in the sex ratio of Maharashtra state over a period of thirty-two years (1980- 2011) using anonymised routinely collected health care data.

H) Methods

1) Study design

1) For adolescent girls, cross sectional survey will be conducted, while for pregnant women a prospective study will be conducted to see risk of anaemia and also its impact on birth weight.
2) Diagnostic analyses will be performed to evaluate application of the non-invasive haemoglobin technology (NBM 200).
3) For the state sex ratio analysis, longitudinal data analysis approach will be used.

2) Study settings and population

The study will be conducted in 35 to 40 villages within the project area of HMF and cover a population of about 65,000. We expect approximately 2500 adolescent females (in total) and 650 new pregnancies every year in our overall study population.

3) Inclusion criteria

Pregnant women:
- A permanent resident of the village specified in the project area
- Confirmed pregnancy from 24th April 2014 to 31st December 2014

Adolescent females:
- Unmarried girls aged between 13-17 years
- Resident of the village specified in the research area

Sex ratio:
- Records between 1st January 1980 to 31st December 2011
- Location of birth: Osmanabad or Sholapur district data/ Maharashtra birth registry (included all 33 districts, depending on data availability)

4) **Exposures of interest (see 7, Data collection)**

5) **Outcome data**

A) Anaemia measurements (for pregnant mothers): Maternal anaemia: Anaemia will be measured by using an automated haematology analyser (Sysmex XP-100, Japan). For pregnant women, this will be measured during the first trimester. Only one measurement will be recorded. The machine will provide following parameters.

General blood
1. White blood cells (WBC)
2. Red blood cells (RBC)
3. Haemoglobin (HGB)
4. Haematocrit test (HCT)
5. Mean corpuscular volume (MCV)
6. Mean corpuscular haemoglobin (MCH)
7. Mean corpuscular haemoglobin concentration (MCHC)
8. Platelet count (PLT)

WBC differential: Screening of inflammation
1. Lymphocytes (LYM)
2. Neutrophils (NEUT)
3. Mixed (monocytes, basophils, eosinophil)

Screening for anaemia

1. Red cell distribution- standard distribution (RDW-SD)
2. Red cell distribution- coefficient variation (RDW-CV)

Screening for the cause of thrombocytopenia

These parameters have sufficient sensitivity and specificity to diagnosis immune thrombocytopenia (17).

1. Platelet size deviation width (PDW)
2. Mean platelet volume (MPV)
3. Platelet large cell ratio (P-LCR)
4. Plateletcrit (PCT)

Non-invasive measurement: Haemoglobin will be also conducted using a non-invasive device NBM 200 to validate the technology in rural Indian women. All referrals will be based on the gold standard measurement (Sysmex XP-100).

Pregnancy and neonatal health outcomes

1) Delivery outcome (spontaneous abortion/induced abortion/stillbirth/live birth/neonatal and infant death)
2) Birth weight (continuous variable)
3) Low birth weight (yes/no, binary categorical variable)

Definitions

Spontaneous abortion: Death of foetus *in utero* up to 28 weeks of gestation.
Induced abortion: Death of the foetus that is medically or otherwise induced.
Stillbirth: Baby born with no signs of life at or after 28 weeks of gestation.
Live birth: The birth of living foetus regardless of length of gestation.
Infant death: Death of baby within first year from birth.
Neonatal death: Death of new-born within first 28 days of life.
Normal delivery: Natural vaginal delivery between 37 and 42 completed weeks of pregnancy.

Maternal health outcomes

1) Date and place of delivery (hospital/home/other)
2) Method of delivery (vaginal/caesarean)
3) Maternal death during pregnancy or during delivery (yes/no and date of death)

B) Adolescent girl data: For adolescent girls Sahli's hemometer will be used to conduct test at field level. Along with this method the NBM 200 will also be used. All referrals and will be based on Sahli's results.

Outcomes

Haemoglobin level (as a continuous measure)
Anaemia (yes/no, binary categorical variable)

C) Sex ratio data (for data from local hospitals/government 1st January 1980- 31st December 2011)

- Number of male live births in each year
- Number of female live births in each year

6) Sample Size Calculation

As this is a feasibility study within the population covered by HMF's health work, there is a fixed number of individuals in the study population, so we have not conducted a formal sample size calculation. For adolescent girls first 1000 girls will be recruited. The recruitment will start from a village with least total population and then will be progressed in an ascending order. The recruitment will stop after achieving complete data from a minimum of 1000 girls. For pregnant women, all identified pregnancies in the outlined period will be eligible to participate. Based on HMF's experience, we expect about 325 pregnancies in recruitment period.

7) Data Collection

Recent research (15,16) suggests that haemoglobin estimation alone is not sufficient to assess body iron reserves especially during pregnancy, and many other blood parameters are likely to be overlooked. The use of cell counter machines is recommended to achieve maximum accuracy to identify anaemia. Therefore, this study will use cell counter machine by a qualified medical technician in pregnant women participants. Other measurements such as blood pressure measurement, diabetes tests (Using biochemistry analyser**)**, height and weight measurements will also be obtained from all study participants. A detailed medical case history for each study participant will be taken during the field visit. Medical case histories and blood investigation reports will be made available to participants and any abnormal reports or cases of clinical concern will be referred to the HMF hospital. The data collection will be piloted before widespread implementation and modified as a consequence of this experience.

Objective one: *To identify individual and village level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 35 villages of Osmanabad district, Maharashtra using an observational epidemiological survey.*

Data collection on risk factors of anaemia will be grouped chiefly into individual level risk factors and village level risk factors. Data collection sheets will also include a consent form and on completion of an interview, collected information will be shared with each participant in the form of a patient record. Basic demographics will be collected for each participant. Interviews will be conducted in the local language (Marathi), which will be then entered electronically into Microsoft Excel in English.

Individual risk factors for anaemia

Basic demographics: Date of entry of data (DD/MM/YYYY)

Name, Age (years), Date of Birth (DD/MM/YYYY if available), Marital status (Yes/No), Age at marriage (years, if known), Education (primary education: until 4^{th} standard, secondary education: until 7^{th} standard, high school: until 10^{th}, higher secondary education: until 12^{th} standard, Graduate, Postgraduate), Occupation, Job details (employed in other company/ self-employed/farming/not working), Monthly income (per month income in Indian Rupees), Caste (open category/ reserve category/ other/not known), Family status (Below Poverty Line/ Above Poverty Line), Family holds yellow card (Yes/No, indicator of Below Poverty Status), Number of children in family, Number of children, House structure (temporary/semi-permanent/permanent) Farming land (in acres), Water pump at farm (yes/no), Vehicle owned: Two wheeler (Yes/No), Four Wheeler (Yes/No), Family income (per month if

known), Telephone/Mobile available (Yes/No, if yes then mobile number), Number of cows, Number of buffalos, Number of goats/sheep.

Health data: Height (cm), Weight (kg), Mid arm circumference (cm), Body Mass Index, Blood pressure, Blood investigation (cell counter machine), History of any surgical procedures, Other diseases (Hypertension/Diabetes/Tuberculosis/Epilepsy/Renal diseases/Not known), Disease notes: (note important findings if not mentioned above), Current medication (if any), Vaccination status (Toxoid tetanus/other: note).

(Height, weight and mid-arm circumference will be recorded in the presence of the primary investigator AA during field visit for data collection, and equipment will be supplied by HMF. Weight will be taken using digital scales for maximum accuracy, height mid-arm circumference will be measured using high quality measuring tapes. For the purpose of blood investigation, sterile disposable needles will be used for collection with samples stored in EDTA bottles with strict follow-up of aseptic protocol. These bottles will be labelled with a unique patient identification number and carried in recommended boxes for investigation at hospital. Blood investigations will be conducted using cell counter at the foundation's hospital and printed reports will be attached with the data collection form. List of investigation parameters is outlined previously. HMF hospital will provide lab technician on a permanent basis during field visit. Results will be shared with patients using telephone communication or through village health worker wherever appropriate).

(For adolescent girls Hb test will be conducted at village level using Sahli's hemometer and reports will be given immediately followed by counselling).

Any identified cases with severe anaemia/ any other emergencies will be referred and documented. All data collection will be planned and processed only in physical presence of AA.

Obstetric history (not applicable for adolescent girls): 1) Stillbirth 2) Spontaneous/induced abortions 3) Live children 4) Hospital visits for obstetric issues (month/year) 5) Previous delivery setting (Not applicable/Hospital/Home) 7) Spacing between deliveries: 1st delivery/abortion (date), 2nd delivery/abortion (date), 3rd delivery/abortion (date), Spacing between 1st and 2nd: year and month, Spacing between 2nd and 3rd: year and month.

Dietary intake

Vegetarian food (per typical week): (1) Green leafy vegetables (2) Pulses (3) Milk (3) Rice (4) Fruits

Non-Vegetarian food (per typical week): (4) Eggs (5) Chicken (6) Meat/Fish

Knowledge about health

- Have you heard about anaemia?
- Have you heard about haemoglobin?
- Have you undergone any tests for haemoglobin?
- Have you taken iron supplements/vitamins before?
- If yes to supplements, when (if known), how many (if known), and how did you obtain them?
- Have you heard about family planning methods?
- What contraceptive methods have you heard of? Do you use them?

Village risk factors for anaemia

Village level risk factors (information will be collected from HMF's village health workers, who are permanent residents of each village and work within the existing government healthcare infrastructure):

Healthcare provision: Government health centre available in village (Yes/No), Government Nurse (Residing/Visiting), Nurse services (Regular: at least once a month/ Not regular), Availability at village level (Blood pressure measuring unit in village, Nurse services (Blood pressure check-up [Yes/No]/ haemoglobin test [Yes/No] /Iron supplements [Yes/No]/ Vitamins supplements [Yes/No]/ Vaccination [Yes/No]), Other private doctor available (Yes/No), Private doctor (Residing/Visiting)
Transport services: Government public transport available (Yes/No), Frequency 24/7 (Yes/No), Duration available, Government ambulance service (Yes/No), Private transport at village (Yes/No), Type of private transport (three wheeler/four wheeler), Frequency 24/7 (Yes/No), Distance from National Highway No 9 (nearest highway), Distance from nearest government hospital for pregnancy emergency, Travel time required to the nearest government hospital for pregnancy emergency, Road type (permanent/ semi-permanent/ unpaved road)

Objective three: *To study temporal changes in the sex ratio over a period of thirty-two years (1980- 2011) in Osmanabad and Sholapur districts (or Maharashtra state data deepening on availability) using anonymised routinely collected health care data.*

Information on sex from registered live births will be collected from hospitals in the Sholapur and Osmanabad districts/ government data collection facility. If available, then data will be collected from birth registry, which will have all births (hospital or home) in specified duration.

7) Data analysis
Statistical analysis will be conducted using Stata software. Descriptive statistics will be reported with percentages (**%)**, frequencies, means and standard deviations. Data analysis plans for each study objective are outlined below:

Objective one: *To identify individual and village level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 35 villages of Osmanabad district, of Maharashtra using an observational epidemiological survey.*

Details about basic demographics will be analysed through categorical variables and wherever applicable continuous variables will be used to optimise the efficiency of the analysis. The haemoglobin measures will be used to generate prevalence of anaemia using a cut off of 11 g/dl in pregnant women (1). Adolescent girls will be assessed using a cut off of 12 g/dl. Further risk factors such as socioeconomic status, caste, age, parity and dietary habits will be evaluated using logistic regression with anaemia as the outcome. Blood investigations will be compared with obstetric history and presented as odds ratios or continuous outcomes where appropriate. We will also explore the prevalence of diabetes and hypertension and risk factors for these diseases using the exposure data collected.

Objective two: *To study temporal changes in the sex ratio over a period of thirty-two years (1980-2011) in Osmanabad and Sholapur districts (or state birth registry) using anonymised routinely collected health care data.*

A time-series design/other suitable approach will be employed. The yearly number of male and female births for 32 years (1980-2011) will be used to investigate any change in sex ratio in these two districts. In the year 1994, the government of India passed legislation banning the use of ultrasound for sex identification with additional amendments in 2002. This was done to prevent the use of diagnostic techniques for foetal sex identification as well as sex selective abortions. We will not be collecting any information on foeticide directly.

I) Limitations

This study will be carried out within a specific geographic area and therefore the findings may not be generalisable to culturally different populations based in India. Furthermore, village level risk factors of acquiring anaemia could be different in urban areas compared to those in rural sector**.**

J) Ethical considerations

A written consent form available in local language (Marathi) will be obtained from study participants before enrolling in research. The form will be explained by a trained staff member/ the investigator involved and oral consent will be recorded on behalf of illiterate participants by project staff in the presence of HMF healthcare worker/family member who will act as a signatory. The consent form will be attached to the data collection sheets. In this research, there is no drug administration, treatment or medical supervision. Study participants will be involved through interviews and a blood test as outlined above. Blood investigation results will be shared with study participants on completion. Observed abnormal reports and cases will be referred to the hospital for expert opinion. There is no financial incentive for study participants.

K) Research setting

Research will be conducted in two blocks as outlined previously (Tuljapur and Lohara blocks of Osmanabad district). In each village the responsible HMF village healthcare worker will be involved during the field activity. The HMF hospital is a secondary care centre and provides diagnostic services especially for pregnant women and will be used for diagnostic services required during this research. In addition, the hospital will provide laboratory technicians and staff whenever required during the programme. Fieldwork will be conducted by PhD student (AA) with the support of data assistants/HMF healthcare worker in each village. Data management such as data entry to computer systems, data cleaning will be conducted at HMF. Data will be handled only by AA and later will be shared with project supervisors in the UK.

L) Data confidentiality and Storage

Research data will be anonymised and will be stored in a password-protected folder in a secure computer drive. The anonymised data will be also saved on a secure drive (R drive: HaloData folder) of University of Nottingham, which allows access to only approved login accounts working in this project (PhD student and PhD supervisors). The University of Nottingham stores data in backup servers for a period of seven years.

M) Dissemination of research findings

Results from this research will be submitted to peer-reviewed journals and will be also presented at international conferences.

N) References

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Appendix 4: Ethical approvals

1. Ethical clearance received from the University of Nottingham, UK



The University of Nottingham

Direct line/e-mail
+44 (0) 115 8232561
Louise.Sabir@nottingham.ac.uk

Faculty of Medicine and Health Sciences

Research Ethics Committee
Division of Respiratory Medicine
D Floor, South Block
Queen's Medical Centre
Nottingham University Hospitals
Nottingham
NG7 2UH

1st November 2013

Dr Anand Ahankari
PhD Student
Division of Epidemiology & Public Health
B124, Clinical Sciences Building
City Hospital Campus
Nottingham University Hospitals
Hucknall Road
Nottingham
NG5 1PB

Dear Dr Anankari

Ethics Reference No: E10102013 SoM EPH – please always quote.
Study Title: An investigation of factors associated with adolescent health and pregnancy-related outcomes in women from Maharashtra state India.
Academic Supervisors: Dr Andrew Fogarty, Clinical Associate Professor, Dr Laila Tata, Associate Professor in Epidemiology, Dr Puja Myles, Associate Professor, Division of Epidemiology and Public Health, School of Medicine
Student Investigators: Dr Anand Ahankari, PhD Student, Division of Epidemiology and Public Health, School of Medicine
Duration of Study: 01/01/2014-31/03/2017, 3yrs 3 mths **No of Subjects:** 40 villages

Thank you for your recent application which was considered by the Committee at its meeting on 10th October 2013 and the following documents were received:

1. UoN FMHS Med Sch Research Ethics Application form dated 26/9/2013.
2. Detailed Research Protocol, version 1.0, dated 26/9/2013.
3. Appendix A Permission letter from Dr Kranti Rayamane, Join Secretary Halo Medical Foundation, India dated 29th July 2013
4. Appendix B Research Highlights dated 29th July 2013
5. Appendix C List of Villages and details of data collection 26/9/2013
6. Appendix D Approval from local Ethics Committee: Institutional Ethics Committee, Gov .Medical College, Aurangabad-431001 Dr SM Doifode, Member Secretary, dated 25th September 2013.
7. Appendix E Contact details in India
8. Appendix F: Participant Information Sheet version 1.0, 26/9/2013
9. Consent Form, version 1.0, 26/9/2013
10. Action plan for referral version 1.0 dated 1/11/2013.

These have been reviewed and are satisfactory and the study is approved.

Approval is given on the understanding that the Conditions of Approval set out below are followed.

1. A Favourable opinion is given on the understanding that all appropriate ethical and regulatory permissions are respected and followed in accordance with all

The University of Nottingham

local laws of the country in which the study is being conducted and those required by the host organisation/s involved.

2. You must follow the protocol agreed and inform the Committee of any changes using a notification of amendment form (please request a form).

3. You must notify the Chair of any serious or unexpected event.

4. This study is approved for the period of active recruitment requested. The Committee also provides a further 5 year approval for any necessary work to be performed on the study which may arise in the process of publication and peer review.

5. An End of Project Progress Report is completed and returned when the study has finished (Please request a form).

Yours sincerely

pp

Dr Caroline Chapman
Acting Chair, Nottingham University Medical School Research Ethics Committee

2. Ethical certificate received from Government Medical College, India

INSTITUTIONAL ETHICS COMMITTEE

GOVT. MEDICAL COLLEGE, AURANGABAD -431001

Phone: (0240) 2402412-17 - EXT.274 Fax No. (0240) 2402418/19

Office: Member Secretary, IEC – Professor & Head, Department of Pharmacology, Govt. Medical College, Aurangabad.

No : Pharma/IEC-GMCA/ 331 /2013 Dated : 25-Sep-2013

To,

DR. ANAND AHANKARI
Project Co-ordinator,
HALO Medical Foundation,
India.

Dear Doctor,

The Institutional Ethics Committee of Govt. Medical College and Hospital, Aurangabad reviewed and discussed your Application to conduct the clinical trial entitled **"AN INVESTIGATION OF FACTORS ASSOCIATED WITH ADOLESCENT HEALTH AND PREGNANCY-RELATED OUTCOMES IN WOMEN FROM MAHARASHTRA, INDIA"** in IEC meeting on 25th September 2013.

The following documents were reviewed:

1. Trial Protocol.
2. Case report form.
3. Patient Information Sheet and Informed Consent.
4. Proposed methods for patient accrual.
5. Principal Investigator's current CV.
6. Investigator's undertaking.

The following members of the ethics committee were present at the meeting.

1. Chairperson (Clinician) Dr. S. H. Talib (MD Medicine) Professor of Medicine MGM Medical college, Aurangabad.	**5. Member (Clinician-Surgeon)** Dr. Ajay Boralkar (MS Surgery) Associate Professor Surgery Govt. Medical College, Aurangabad.
2. Member Secretary (Pharmacologist) Dr. S M Doifode(MD Pharmacology) Prof & HOD of Pharmacology Govt. Medical College, Aurangabad.	**6. Member (Basic Medical Scientist)** Dr M S Baig (MD Pharmacology) Assistant Professor of Pharmacology Govt. Medical college, Aurangabad
3. Member (Outside Clinician) Dr. Sudhir Kulkarni (MD Medicine) Professor of Nephrology. MGM Medical college, Aurangabad.	**7. Member (Legal Expert)** Mrs B V Paranjape (LL.B) Assistant Professor MP Law College, Aurangabad
4. Member (Basic Medical Scientist) Dr. K U Zine (MD FMT) Associate Professor & Head of FMT Govt. Medical College, Aurangabad.	**8. Member (Lay person)** Shri A P Dhaneshwar (BA) Deshpande puram, Shahnoorwade, Aurangabad.

We **approve** the trial to be conducted in its presented form. The Institutional Ethics Committee expects to be informed about the progress of the study, any changes in the protocol and patient information / Informed consent and asks to be provided a copy of the final report.

Member Secretary,
Institutional Ethical Committee,
Govt. Medical College, Aurangabad

(4)

Appendix 5: Scholarship and bursary grants awarded to Dr Anand Ahankari (PhD candidate)

All successful project grants and scholarships awarded in the recent five years are outlined below.

- Personal education/scholarship grants awarded: **57,600 GBP**
- Total project grants secured: **375,630 GBP**

Title	Source	Amount	Details	Role
Mr Hendry Postgraduate Research Tri Campus Award of 2016	University of Nottingham, UK	Felicitation and 150 GBP	The award was given following the contribution made during PhD studies. The selection was made through a formal assessment of all final year PhD students across medicine faculty of the University of Nottingham UK.	Awardee
Travel award, January 2016	Graduate school and Epidemiology division University of Nottingham, UK	750 GBP	Conference registration and travel costs. Women Delivery 2016 Copenhagen, Denmark.	Awardee
Travel award, June 2015	Epidemiology division, University of Nottingham, UK	300 GBP	Academic visit to the Public Health Foundation of India (PHFI), New Delhi.	Awardee
Gender equity project, May 2015	Maharashtra Foundation, USA	4,000 GBP	Project grant to Halo Medical Foundation (HMF), India to implement gender equity programme in Sholapur city urban slums	Primary Applicant
Bursary scholarship, April 2015	Durgadevi Charitable Trust, India	4,000 GBP	PhD education support	Awardee
Hospital outreach programme, February 2015	Bitwise Solutions Pvt Ltd India	800 GBP	Project grant to HMF hospital, India	Primary Applicant
HMF Trauma Centre expansion grant, May 2014	Goldman Sachs Gives (GSG), UK for Mr Gupta's CSR funds	100,000 GBP	Advancing trauma centre facilities, equipment and corpus grant to HMF India	Primary Applicant
Research equipment grant, January	Clinical Translational Priority	1,250 GBP	Purchase of non-invasive haemoglobin device for Maharashtra Anaemia Study	Co-applicant

2014	Research Group, University of Nottingham, UK		(MAS)	
Pregnancy buy-a-bed programme, December 2013	Bitwise Solutions Pvt Ltd India	1,200 GBP	Project Grant to HMF hospital, India.	Primary Applicant
Maharashtra Anaemia Study, September 2013	Maharashtra Foundation, USA through charity donation of Dr Hardikar, Texas.	15,000 GBP	Project Grant to HMF India to implement MAS study in collaboration with the University of Nottingham, UK	Primary Applicant
Vice Chancellor Scholarship for Research Excellence, May 2013	University of Nottingham, UK	38,490 GBP	100% international tuition fee support for PhD studies at the University of Nottingham, UK	Awardee
HMF Trauma Centre establishment grant, February 2013	Goldman Sachs Gives, UK for Mr Gupta's CSR funds	226,580 GBP	Building construction and hospital development grant to HMF India	Primary Applicant
Travel award, November 2011	School of Medicine, University of Nottingham UK	1,000 GBP	Poster presentation at the 19th ICOWH congress, Thailand.	Awardee
Hospital equipment grant, March 2012	Cognizant Foundation, India	12,000 GBP	Equipment grant to HMF hospital, India	Co-applicant
Hospital equipment grant, July 2011	Indicorps, USA	5,000 GBP	Equipment grant to HMF hospital, India	Co-applicant
Bursary Scholarship, June 2011	Durgadevi Charitable Trust, India	3,500 GBP	MPH tuition fees support	Awardee
Developing Solution Masters Scholarship, May 2011	University of Nottingham, UK	9,350 GBP	50% international tuition fee support for MPH studies at the University of Nottingham, UK	Awardee
Community leadership	Toyota Foundation,	11,000 GBP	Project grant to HMF to implement health leadership	Co-applicant

programme to improve maternal health outcomes by promoting leadership in urban slums, March 2011	Japan		programme in Sholapur city urban slums	
Travel award, June 2010	SBP Dental College, India	100 GBP	Cost towards conference registration at international dental symposium in Hong Kong	Awardee
Merit student award 2007, 2008, 2009	SBP Dental College, India	Felicitation	Merit student award for securing top rank during undergraduate studies	Awardee

Appendix 6: Data collection forms

1. Data collection form for adolescent girls

With the support of Halo Medical Foundation, India and the University of Nottingham, UK

An investigation of factors associated with adolescent health outcomes in Maharashtra, India. **AG**

Dear Participant
We are looking to improve the health of adolescent girls by identifying risk factors that lead to poor health. The work involves the collection of information through this questionnaire and a simple haemoglobin test performed by a qualified technician. We will share your haemoglobin report with you. Your information and reports will be totally confidential and will only be seen by appropriate professionals involved in this study. Results of this research will be used for journal publication or presented at conferences, however it will not be possible to identify individual from this. The details of the project are outlined below. We would like to invite you to participate in this study. Please read all details and if you agree to participate please provide signatures in the allotted space in the presence of your parents/legal guardian, who will countersign the form.

Participant information

- *Project title:* An investigation of factors associated with adolescent health outcomes in Maharashtra state, India.
- *Purpose of this data collection:* To identify risk factors for poor health in adolescent girls. You will be eligible to participate in this study if you are 13 to 17 years old.
- *Study procedure:* Interview and a haemoglobin test, which involves a finger prick.
- *Project period:* September 2013 – August 2017
- *Risks:* There is no intervention or drug or medication proposed and hence no risks are anticipated.
- *Possible benefits:* Haemoglobin report at no cost with referrals to nurse/hospital if indicated by results.

Confidentiality: If you agree to participate in this study, the information obtained will be kept confidential and only relevant authorities such as the Ethics Committee will have access to the records. Medical records will be shared with doctors and health care professionals only for the purposes of your healthcare and will otherwise be treated as confidential. Similarly, any published reports will only include non-identifiable information.
Voluntary participation Your participation in this study is entirely voluntary. You may refuse to take part in this study. You may withdraw at any time.

Questions: You are encouraged to ask questions and clear any doubts. If you need further information about the study or would like to discuss in detail please do not hesitate to contact: Dr Anand Ahankari, Halo Medical Foundation, At Post: Andur, Block: Tuljapur, Dist: Osmanabad, Maharashtra, India 413603. Phone: +91-7798616776.

Signature page- I confirm and understand that:

1. I have read the participant information outlined above and then carefully considered my participation in this research.
2. I have had a chance to ask questions and these have been answered to my satisfaction.
3. My participation in this study is entirely voluntary.
4. I have had the chance to discuss my participation in this research with my parents/local guardian.
5. I agree to follow study procedures and provide information as required by the investigating team, of my own free will.
6. I am aware that I am free to withdraw at any time from the study.
7. I have received a copy of the participant information sheet for my own record.
8. My medical records will be shared with doctors and health care professionals only for the purposes of my healthcare and will otherwise be treated, as confidential. Only non-identifiable data will be published for research purposes.
9. I allow the investigator at the Halo Medical Foundation and University of Nottingham, UK to use any data or results arising from this study for analysis and publication.
10. I…… exercising free power of my choice give my consent to be included in this study. I also agree to undergo examination and investigation for this research. I have received all study information and I am participating of my own choice.

Participant's name: _____first name/middle name/surname) Agreed to participate Yes/No: _____

Signature or if participant cannot sign then impression of left thumb: _____
Address: At Post: _____Block: Tuljapur ( )/Lohara ( ),
Dist: Osmanabad

Name of local guardian/ mother/father: _____
Signature or if they cannot sign then impression of left thumb: _____
Phone Number: _____
Address: At Post: _____Block: Tuljapur ( )/Lohara ( )
Dist: Osmanabad

Name of data entry assistant: _____Sign: _____

Name of principal investigator: Dr Anand Ahankari
On completion of data collection, tests and checking for requirement for medical referrals sign here: _____ Date: __ __ /__ __ /__ __

Date of data collection: _ _ /_ _ /_ _ _ __ Unique identification number: _____

Participant Information (Use abbreviations Y = Yes, N =No)

No	Question	Answer	
1	Age (only girls between 13 to 17 years are eligible to participate in this research)	_____ Years (completed)	Eligible: Y or N
2	Date of birth (if not available write NA)	_ _ /_ _ / _ _ _ _ (DD/ MM/ YYYY)	
3	Are you attending school/college?	Y or N	
4	Are you doing any paid work outside your family (within farm/factory/shop)?	Y or N	
5	How many older living siblings do you have?	Brothers	Sisters
6	How many younger living siblings do you have?	Brothers	Sisters
7	Height (in centimetres only)	_____ cm	
8	Weight (in kilograms only)	_____ kg	
9	Mid-arm circumference (dominant hand) (in centimetres only)	_____ cm	Right Hand: ( ) Left Hand: ( )
10	Do you have any diagnosed disease at the moment? (diabetes, epilepsy, tuberculosis or other please specify) If yes then write the disease name	Y or N Name of disease	
11	Have you ever taken iron folic acid tablets in the past? (DN: Do not know)	Y or N or DN	
12	Are you currently taking iron folic acid tablets?	Y or N or DN DN then directly go to 14	If N o
13	Who is providing your iron folic acid tablets? (A) School (B) Government	A or B or C or D (you can choose multiple answers here	If D, then specify _____

	nurse (C) Family bought (D) Other, then specify		_____
14	Are you currently taking calcium tablets?	Y or N If N then directly go to 16	
15	Who is providing your calcium tablets? (A) School (B) Government nurse (C) Family bought (D) Other, then specify	A or B or C or D (you can choose multiple answers here)	If D, then specify _____ _____
16	Are you taking any medicines at the moment including multivitamins? Please write the exact name as on tablets/bottles	Y or N Name of medicine	
17	Which category do you think you belong to? (Please CIRCLE only one option) (A) Hindu open category (B) Hindu reserve category (SC/ST/OBC) (C) Muslim open category (D) Muslim reserve category (OBC) (E) Other religion, please state _____ (F) Declined to answer		
18	How would you describe your house structure? A) Temporary (Kaccha) B) Semi-permanent (Semi-Pucca) C) Permanent (Pucca)	A or B or C	
19	How much farming land do your parents own? (A)None (B) Less than 5 acres (C)More than 5 acres	A or B or C	
20	Do your parents own any livestock? A) Yes B) No	Y or N If N then go to 22 directly	
21	If yes, then how many livestock do your parents have? Put numbers in the relevant box	**Livestock**	**Total Number**
		Cows	
		Buffalo Bulls	
		Sheep/Goat	
		Chickens	
		Other (name_____)	
22	Do your parents own a television set?	Y or N	

23	Do your parents own a mobile phone?	Y or N
24	Is your family registered with the Below Poverty Line list?	Y or N
25	Which type of food do you eat? A)Vegetarian (without egg) B)Vegetarian (with egg) C)Non-Vegetarian	A or B or C

26 Could you tell us more about your specific food intake over the last week (last seven days of diet)?

Write Y in appropriate the boxes

Food item	**Everyday**	**3-6 days a week**	**1-2 days a week**	**Never**
a) Milk				
b) Green leafy vegetables				
c) Sprouts				
d) Pulses				
e) Fruits/Juices				
f) Rice				
g) Egg				
h) Chicken				
i) Mutton/Meat				

27	Have you ever heard about anaemia and haemoglobin tests?	Y or N
28	Have you had your haemoglobin checked before? (DN do not know)	Y or N or DN (do not know)
29	Have you been told before that you are anaemic? (DN do not know)	Y or N or DN (do not know)
30	Have you started having your menstrual periods? Write D if Declined to answer	Y or N or D If N or D, then go to 34
31	How old were you when you had your first period? Write D if Declined to answer	_____Years (D/DN do not know) Month and year when participant shad first menstrual cycle
32	Have you ever missed school in the past 12 months due to your periods? Write D if Declined to answer	Y or N or D If N or D, then go to 34

33	If yes, then how many days have you missed in the past 12 months? Write D if Declined to answer	_____ Days (D/DN do not know)	
Haemoglobin test details, for the use of technician and primary investigator (Use ring finger of non-dominant hand)			
34	Haemoglobin test performed	Y or N, If N write reason	
35	Haemoglobin reading	_____ g/dl	First name of technician
36	Was there any complication as a result of the test? If yes then write notes on a separate paper	Y or N	
37 38 39	Is the participant anaemic? (Haemoglobin < 12.0 g/dl, then anemic) Haemoglobin reading by non-invasive electronic machine Was there any complication or error of the test with electronic machine?	Y or N _____ g/dl Y or N	
40	If Yes, have you given a referral card to the participant?	Y or N	
End of Data Collection			

2. Data collection form for Pregnant Women Participants

With the support of Halo Medical Foundation, India and the University of Nottingham, UK

An investigation of factors associated with pregnancy-related outcomes in women from Maharashtra state, India. **PW**

Dear Participant

We are looking to improve the health of pregnant women by identifying risk factors that lead to poor health. The work involves the collection of information through this questionnaire and a blood test performed by a qualified technician. We will share your blood reports with you. Your information and reports will be totally confidential and will be seen only by appropriate professionals and researchers involved in this study. Results of this research will be used for journal publication or presented at conferences; however it will not be possible to identify individuals from this. The details of the project are outlined below. We would like to invite you to participate in this study. Please read all details and if you agree to participate then provide signatures in the allotted space in the presence of a witness, who will countersign the form.

Participant information

Project title: An investigation of factors associated with pregnancy-related outcomes in women from Maharashtra state, India.

Purpose of the study: To identify risk factors for poor health during pregnancy.

Study procedure: Interview and blood test. You will be eligible to participate in this study only if you are between 3 to 5 months of pregnancy.

Project period: September 2013 – August 2017.

Risks: There is no intervention or drug or medication proposed and hence no risks are anticipated.

Possible benefits: A blood investigation report at no cost with referrals to hospital if indicated by results.

Confidentiality: If you agree to participate in this study, the information obtained will be kept confidential and only relevant authorities such as the Ethics Committee can have access to the records. Medical records will be shared with doctors and health care professionals only for the purposes of your healthcare and will otherwise be treated as confidential. Similarly, any published reports will only include non-identifiable information.

Voluntary participation Your participation in this study is entirely voluntary. You may refuse to take part in this study. You may withdraw anytime.

Questions: You are encouraged to ask questions and clear any doubts. If you need further information about the study or would like to discuss in detail please do not hesitate to contact: Dr Anand Ahankari, Halo Medical Foundation, At Post: Andur, Block: Tuljapur, Dist: Osmanabad, Maharashtra, India 413603. Phone: +91-7798616776.

Signature page I confirm and understand that:
I have read the participant information sheet and then carefully considered my participation in this research.
I have had a chance to ask questions and these have been answered to my satisfaction.
My participation in this study is entirely voluntary.
I agree to follow study procedures and provide information as required by the investigator team, of my own free will.
I am aware that I am free to withdraw at any time from the study.
I have received a copy of the participant information sheet for my own record.
My medical records will be shared with doctors and health care professionals only for the purposes of my healthcare and will otherwise be treated as confidential. Only non-identifiable data will be published for research purposes.
I allow the investigator and researchers at the Halo Medical Foundation and University of Nottingham, UK to use any data or results arising from this study for analysis and publication.
I…… exercising free power of my choice give my consent to be included in this study. I also agree to undergo examination and investigation for this research. I have received all study information and I am participating of my own choice.

Participant's name: _____ (first name/middle name/surname)

Agreed to participate Yes/No: _____ Phone Number: _____

Signature or if participant cannot sign then impression of left thumb: _____
Address: At Post: _____
Block: Tuljapur ( )/Lohara ( ) Dist: Osmanabad

Name of witness: _____
Signature or if they cannot sign then impression of left thumb: _____
Address: At Post: _____
Block: Tuljapur ( )/Lohara ( ) Dist: Osmanabad

Name of data entry assistant: _____Sign: _____

Name of principal investigator: Dr Anand Ahankari

On completion of data collection, tests and checking for requirement for medical referrals sign here: _____ Date: __ __ /__ __ /__ __ __ __

Date of data collection: _ _ /_ _ /_ _ _ __ Unique identification number: _____

Participant Information (Use abbreviations Y = Yes, N= No, D= Declined to answer)

No	Question	Answer		
1	How many months have you been pregnant?	_____ month (must be between 3-5 months only)		
2	Age	_____ Years (completed)		
3	Date of birth Write NA (not available) if not available	_ _ /_ _ / _ _ _ _ (DD/MM/YYYY format)		
4	Marital status A) Married B) Unmarried C) Widow	A or B or C		
5	Age at marriage	_____ Years		
6	Marriage within the family (to cousin)	Y or N		
7	Education (circle one option only) (A)Less than Secondary School Certificate (< 10th class) (B) Secondary School Certificate (10th class) (C)Higher Secondary School Certificate (12th class) (D) Graduate and further qualifications			
8	Do you know the approximate date of your last menstrual period? If participant is unaware then write NA (not available)	_ _ / _ _ / _ _ _ _ (DD/ MM/ YYYY)		
9	Height (in centimetres only)	_____ cm		
10	Weight (in kilograms only)	_____ kg		
11	Mid-arm circumference (dominar hand)	_____ cm	Right Hand: ( ) Left hand: ( )	
12	Sitting blood pressure (mm Hg) • Two minute interval between consecutive readings. • Take minimum of two readings. If the difference between first two readings of	**Reading count**	**Systolic BP**	**Diastolic BP**
		First reading		
		Second reading		
		Third reading		
		Fourth reading		
		Fifth reading		

	systolic blood pressure is more than 5 mm of Hg, then take further readings until difference of two consecutive readings is ≤ 5 mm of Hg. • Use the average of these two most recent readings to generate the final blood pressure. If more than five readings are required then use space provided at the end of page 6 of this form. See Standard Operating Procedures if required.	**Final average reading** (of two most recent readings)		
13	For the use of primary investigator, Referral Notes (If final blood pressure is ≥140-90 mm of Hg):			
14	Do you have a diagnosis of diabetes?	Y or N	If yes, are you taking any treatment? Y or N	
15	Do you have any diagnosed disease at the moment? (Epilepsy, Tuberculosis or other please specify). If Y, then write the disease	Y or N If Y, then disease name		
16	Have you been taking iron folic acid tablets during any of the following periods? A) Before pregnancy B) After confirmation of pregnancy C) Not taking tablets	A or B or C	If C then19	
17	If you started taking iron folic acid tablets during pregnancy, which month did you start?	_____ month		
18	If you took iron folic acid before or during pregnancy, who prescribed the tablets? A) At government hospital B) Local healthcare worker in village C) Not prescribed, bought from pharmacy D) Other, then specify	A or B or C or D	If D then specify below	

19	Have you been taking calcium tablets during any of the following periods? A) Before pregnancy B) After confirmation of pregnancy C) Not taking tablets	A or B or C If C then go 22	
20	Which month of pregnancy did you start calcium tablets?	_____ month	
21	If yes, then who prescribed the calcium tablets? A) At government hospital B) Local healthcare worker in village C) Not prescribed, bought from pharmacy D) Other, then specify	A or B or C or D	If D, then specify below
22	Are you currently taking any medications including multivitamins? Please write the exact name as on tablets/bottles	Y or N	
		If Y, then medicine name	
23	How many children have you given birth to that were <u>alive</u> at birth (i.e. they were born alive)?	Number	
24	How many children have you given birth to that were <u>NOT alive</u> at birth (i.e. they were stillborn)?	Number	Natural abortion: ( ) Induced abortion: ( )
25	How many children do you have that are alive now and what are their ages?	**Number of boys**	**Number of girls**
		Age B1 = Years	Age G1= Years
		Age B2= Years	Age G2= Years
		Age B 3= Years	Age G3= Years
If more than 3 boys or girls then use this space for additional data in relation to question (list the age and sex of each child)			

26	Which category do you think you belong to? (Please CIRCLE only one option) (A) Hindu open category (B) Hindu reserve category (SC/ST/OBC) (C) Muslim open category (D) Muslim reserve category (OBC) (E) Other religion, please state _____ (F) Declined to answer		
Questions about you and you husband (immediate family only)			
27	How much money do you and your husband earn annually? A) 0-50,000 rupees B) 50,001-1,00,000 rupees C) 1,00,001 and above	A or B or C	
28	How would you describe your house structure? D) Temporary (Kaccha) E) Semi-permanent (Semi-Pucca) F) Permanent (Pucca)	A or B or C	
29	How much farming land do you and your husband have? (A) None (B) Less than 5 acres (C) More than 5 acres	A or B or C then go to 31	If A
30	Do you or your husband have your own motor pump for farming?	Y or N	
31	Do you or your husband own a two-wheeler?	Y or N	
32 33 34	Do you or your husband own a four-wheeler? Do you or your husband have your own television set? Y or N Do you or your husband have your own mobile phone? Y or N	Y or N	
35	Do you or your husband have livestock? A) Yes B) No	Y or N then go to 37	I
36	If yes, how much livestock do you have? Put numbers in relevant box If other then please specify name	**Type of livestock** Cows Buffalo Bulls Sheep	**Total Number**

		Chickens	
		Other (name_____	
37	Is your family registered within the Below Poverty Line list?	Y or N	
38	Have you or your husband been forced to sell any of your assets (including animal and land) in the past 12 months? (livestock/land/gold/valuables)	Y or N	Item sold:

Further question about your diet and health

39	Which food type do you eat? A)Vegetarian (without egg) B)Vegetarian (with egg) C)Non-Vegetarian	A or B or C

40 Could you tell us more about your specific food intake over the last week (last seven days of diet assuming that it was a typical of your usual diet) Write Y in appropriate the box

Food item	**Everyday**	**3-6 days a week**	**1-2 days a week**	**Never**
a) Milk				
b) Green leafy vegetables				
c) Sprouts				
d) Pulses				
e) Fruits/Juices				
f) Rice				
g) Egg				
h) Chicken				
i) Mutton/Meat				

41	Which type of fuel does your household mainly use? (A) Liquid Petroleum Gas (B) Kerosene (C) Wood/Straw/Animal dung (D) Electric Range (D) Other (then specify)	A or B or C or D or E	If E then specify here

42	Have you ever heard about anaemia and haemoglobin tests?	Y or N	
43	Have you had your haemoglobin checked before?	Y or N	
44	Have you been told that you are anaemic?	Y or N	
45	Have you registered with a government nurse during pregnancy?	Y or N	
46	Have you registered with any private hospital during pregnancy?	Y or N	
47	Have you used any method of family planning in the past?	Y or N	
48	Have you received Tetanus Toxoid (TT) vaccine during pregnancy?	Y or N	If Yes, then write number of injections received
49	Was there any complication as a result of the test? <u>Venous blood withdrawal from non-dominant hand</u>	Y or N	If yes then write notes on a separate paper

Use this space for additional blood pressure records if required. Insert table with headings, readings number as previously shown on page 3

End of data collection, Further use for Primary Investigator only: Pregnancy Outcome

50	Blood tests completed and reports attached	Y or N	Date: __ __ / __ __ /__ __ __ __
51	Reports shared with participant: Glucose and Complete Blood Count (CBC)	Y or N	Date: __ __ / __ __ /__ __ __ __
52	In case of any abnormal reports, briefly list abnormalities and action taken	Y or N	Date: __ __ / __ __ /__ __ __ __
53	Abnormalities and action taken:		
54	Referral notes if participant was advised for any referral	Y or N	Date: __ __ / __ __ /__ __ __ __
55	Referral notes:		
56	Expected date of delivery (as per most recent hospital visit)	_ _ /_ _ / _ _ _ _ (DD/MM/YYYY)	

Post- Delivery Data				
57	Total number of hospital antenatal visits during the pregnancy			
58	Was the woman diagnosed with any disease during her pregnancy such as abnormally high blood pressure, diabetes or other?	Y or N	If Y, then specify _____ _____	
59	What was the pregnancy outcome? A) Spontaneous abortion (miscarriage) B) Induced abortion C) Delivery	A or B or C		
60	If A or B above, then write notes about complication here: If answer is C then only continue further.			
61	Date of delivery	_ _ /_ _ / _ _ _ _ (DD/MM/YYYY format)		
62	Place of delivery 1) Hospital delivery 2) Home delivery	1 or 2		
	Type of delivery A) Normal spontaneous vaginal delivery B) Caesarean C) Forceps delivery/other assisted delivery	A or B or C		
63	If hospital delivery, then distance from village (where labour started)	_____ Kilometers		
	Mode of transport used A) Government vehicle (bus or ambulance) B) Private hired vehicle C) Own vehicle	A or B		
64	Hospital details (write name)			
65	Gestational weeks at delivery (if recorded)	_____ weeks		
66	Preterm delivery	Y or N		
67	Baby details	A	Sex of new born	
		B	Birth weight	kg

	Baby 1 <u>Circle only one option out of D/E/F</u> If F, specify requested details	C	Child name	
		D	Baby was born alive	
		E	Baby was born dead (stillbirth)	
		F	Baby was born alive but died after delivery	
			How many hours/days was the baby alive after delivery?	_____ Hours _____ Days
68	Baby details (Use this section in case of twins only) Baby 2 <u>Circle only one option out of D/E/F</u> If F, specify requested details If triplets, please record third name and birth outcomes at the end of this form (in section 69)	A	Sex of new born	
		B	Birth weight	kg
		C	Child name	
		D	Baby was born alive	
		E	Baby was born dead (stillbirth)	
		F	Baby was born alive but died after delivery	
			How many hours/days was the baby alive after delivery?	_____ Hours _____ Days
69	Any complications of pregnancy (write all if more than one) such as haemorrhage, sepsis/infection/ death of mother/mal-presentation/pregnancy induced hypertension	Put N for no complications		
70 71 72	Write additional notes if required What is the haemoglobin reading with non-invasive electronic machine? Was there any complication or error in the above test?	_____g/dl Y or N		

3. Data collection for Village Profile

With the support of Halo Medical Foundation, India and the University of Nottingham, UK

Project title: An investigation of factors associated with adolescent health and pregnancy-related outcomes in women from Maharashtra state, India

Consent		**VLD**
I	Consent of HMF's Village Health Worker (VHW)	I have received information about this research project and am providing these details of my own free will. I am aware that the information in non-identifiable form could be used for publications or at conferences by the research team from Halo Medical Foundation and the University of Nottingham. I have received a participant information sheet for my own record. Name: _____ Sign: _____ Mobile Number:_____
II	Consent of Nurse, if involved	I have received information about this research project and am providing these details of my own free will. I am aware that this information in non-identifiable form could be used for publications or at conferences by the research team from Halo Medical Foundation and the University of Nottingham. I have received a participant information sheet for my own record. Name: _____ Sign: _____Mobile Number:_____
III	Data assistant (if involved)	Name: Sign here:
IV	Primary investigator	Dr Anand Ahankari __ /__ __ / __ __ __ __ On completion of data collection sign here:

Part	**Particulars**	**Information (Use Y for Yes, N for No)**	
A	Date of Data Collection	_ _ / _ _ / _ _ _ _ (DD/MM/YYYY)	
B	Village Name	At Post: _____ Block: Tuljapur ( )/ Lohara ( ) District Osmanabad	
C	Village Profile	According to the local governance body records	
		Total Population (2011)	
		Total Population (2001)	

		According to the government nurse/VHW records Current total population (2013)		
		1) Do you have a local governance body (Grampanchayat)? If yes then how many members does it have?	Y or N	Number
		2) Do you have any women members in the governance body? If yes then how many women does it have?	Y or N	Number
		3) Do you have a village health and sanitation committee?	Y or N	
		4) Do you have a village education committee?	Y or N	
		5) Do you have a public phone (coin box) in the village?	Y or N	
		6) Do you have a government regulated water purification facility in the village?	Y or N	
		7) Do you have a piped water supply?	Y or N	
		8) Do you have community hand pumps/water tanks for water supply?	Y or N	
		9) Do you have a consistent water supply (everyday) throughout the year?	Y or N	
		10) Do you have Community toilets? If yes, then how many are there in the village?	Y or N	Number
		11) Is there any place in your village where men can purchase alcohol? If yes, how many?	Y or N	Number
		12) How many cases of domestic violence against women were reported to the Halo organisation's self-help groups in the past 12 months in your village?	Number	

		(January 13-December 13)	
D	Health Profile	1) Do you have a government health centre in the village?	Y or N
		2) Do you have an ASHA worker (accredited social health activist) in your village?	Y or N
		3) Do you have a government nurse in village?	Y or N If N then go to 9
		4) If yes, do you have A) Residential nurse B) Only visiting nurse C) Supposed to be residential, but stays in village whenever required If answer is C then write major challenges/issues regarding accommodation of government health staff: 1) 2)	A or B or C
		5) Does your nurse provide antenatal services each month at village level?	Y or N
		6) Do you receive medicines from an assigned nurse?	Y or N
		7) Does your nurse provide haemoglobin tests?	Y or N
		8) Does your nurse measure blood pressure?	Y or N
		9) Are there any qualified private doctors working in your village? If yes, how many?	Y or N / Number
		10) Is there a day and night emergency health service centre in village?	Y or N
		11) How many babies were born in the past 12 months in your village? (January 13-December 13)	
		12) How many pregnancies in the past 12 months did not result in live births in the village?(January 13-December 13)	
E	Transport and Accessibility	1) Do you have government transport (Bus) to the hospital from your village?	Y or N, If N then go to 3

		2) Is it a day and night service (Bus)?	Y or N
		3) Are there any private transport providers available for hire (three/ four wheeler)?	Y or N If N then go to 5
		4) Is private transport accessible day and night?	Y or N
		5) Which is the nearest highway from your village? A)State highway B) National highway No 9	A or B
		6) Distance from the village to the nearest highway	_____ Kilometres
		7) Road accessing the nearest highway A) Unpaved B) Paved C) Permanent	A or B or C
		8) What is the distance from the village to the nearest hospital where babies are delivered? (Write 0 if the hospital is based in the village)	_____ Kilometres If answer is 0 then go to 10
		9) What is the usual travel time by private vehicle to this hospital in good daylight conditions?	_____ Hours ____ Minutes
		10) Do you have an ambulance in your village?	Y or N
		11) Is there any ambulance available on call? If yes, then note the response time from telephonic request to arrival.	Y or N _____Hours ____ Minutes

Appendix 7: Health report card

a. Health Report for Adolescent Girls

Date: __ __ /__ __/__ __ __ __

Name of Participant: Miss

_____

Resident of: _____ Dist: Osmanabad.

Age: _____Years. Height: _____ cm. Weight: _____ Kg. Haemoglobin count: _____ g/dl

This report card is provided on a result of participation in the research study titled, 'An Investigation of factors associated with adverse effect on adolescent health and pregnant women in Maharashtra state, India. This card is for the record of participant.

Referral required: _____ (Yes/No) If haemoglobin is 12.0 g/dl or more then the participant does not need any referral.

If yes, then select option below.

This participant is referred to (select one):

(1) Government nurse/HMF healthcare worker/ ASHA ( ) select this option only if haemoglobin is between 8 g/dl to 11.9 g/dl.

(If haemoglobin is 7.9 g/dl or below, then the participant needs hospital referral)

(2) HMF hospital ( ) (3) Government hospital ( ) (3) Participant preferred another hospital ( )

Information about anaemia and haemoglobin: Anaemia is a type of blood condition in which the number of red blood cells or haemoglobin decreases. Ideally after 13 years of age, haemoglobin should be 12.0 g/dl, and if the value is less than this then the individual is anaemic. In India, there are very high numbers of young girls who are affected by anaemia, thus it is very important to consume a healthy diet and iron rich food to have optimum haemoglobin values. One of the iron food items is green leafy vegetables, which is widely available in our area. Please contact your local healthcare worker, government nurse or Halo Medical Foundation's hospital for further advice and information. You can access health education material at free costs from HMF's village health worker in your own village. If you have been referred to the HMF's hospital, then please contact on the following address or visit nearest government hospital. If you have preferred to visit another private or government hospital, then please consult. If you need any advice or wish to discuss more, then do not hesitate to contact on the following address/phone number. Dr Anand Ahankari, Janki Hospital, At Post Andur, Block Tuljapur, Dist Osmanabad, Maharashtra, India 413603 Phone number: Hospital +91-2471-202416

b. Health Report Card for Pregnant Women

Date: __ __ /__ __/__ __ __ __

Name of Participant: Mrs _____
Age: _____Year. Height: _____ cm. Weight: _____ Kg. Participant hypertensive: _____ (Yes/No).
Resident of: _____ Dist: Osmanabad.
CBC report: Attached with this report. Note Main findings_____
Glucose report: Attached with this report. Note Main findings_____

Referral required: _____ (Yes/No).

Participant/Participant family member was communicated after test results and was advised for referral: _____ (Yes/No/Not applicable). (Mention not applicable, if there is no need of referral).

Select the option chosen by participant (after telephonic discussion with participant/participant's family member).
Participant's preference for further follow up/ medical consultation (TICK only one option)

(1) Follow up only- Government nurse/HMF healthcare worker/ ASHA worker ( )
(2) HMF's hospital ( )
(3) Government hospital ( )
(4) Participant is willing to visit another hospital ( )

This report card is provided on a result of participation in the research study titled, 'An Investigation of factors associated with adverse effect on adolescent health and pregnant women in Maharashtra state, India'. This card is for the record of participant.

Contact: Dr Anand Ahankari, Janaki Hospital, At Post Andur, Block Tuljapur, Dist Osmanabad, Maharashtra, India 413603 Phone number: Hospital +91-2471-202416

Appendix 8: Standard operating procedures

Background

Project Title- Maharashtra Anaemia Study: An investigation of factors associated with adolescent health and pregnancy-related outcomes in women from Maharashtra state, India.

Short Title- Maharashtra Anaemia Study (MAS)

Project Location- Tuljapur and Lohara Blocks of Osmanabad district of Maharashtra state, India

Project Partners- Halo Medical Foundation, India and the University of Nottingham, UK.

First Person of Contact: Dr Anand Ahankari
Halo Medical Foundation, At Post Andur, Block Tuljapur, Dist Osmanabad, Maharashtra, India 413603. Email: dr.anandahankari@gmail.com
Phone: Office 0091- 2471- 246384 (10 am to 5 pm Monday to Saturday)
Mobile 0091-7798616776

Document Prepared Between- October 2013- January 2014
Final copy printed on 25th January 2014.

Study team responsibilities

It is important that all members of the study team are aware of their responsibilities to the study participants, in particular:

1. Prompt recognition and action on any abnormal blood pressure, blood glucose, haemoglobin, platelet or neutrophil measurements.
2. Maintenance of confidentiality of personal data and documents or files that may contain these data.

Purpose of the document

The document illustrates the data collection process, the method of recruitment of study participants, guidelines for data collection, conduct of the study and a detailed step by step guide for data entry assistant/investigator. This document should always be carried during fieldwork. It is available in two languages English and Marathi. The 'Standard Operating Procedures' are mandatory to follow for data collection to ensure a uniform method of information collection to maintain the quality during the study period.

Document Translation

Initially all documents of this research project are developed in UK English and then will be made available in Marathi language, which is an official language of Maharashtra state of India. The translation of data collection forms, participant information sheet, health report cards and standard operating procedures will be performed through the involvement of HMF review board member and translation agency, wherever necessary. The translation process will be documented and will be also shared with the research team at Nottingham, UK. Lastly, data collection forms will be back translated into English by a third independent agency and shared with all members involved in the research.

Pilot Process
Data collection forms, adolescent health cards and participant information sheets will be shared with the HMF staff members, consultants and trustees involved in the project. Any recommendations or suggestions will be discussed by the primary investigator and supervisors, and if agreed to be important then will be incorporated into the document. The pilot process will be documented by the primary investigator. Similarly, the standard operating procedures will be shared with the HMF and if any changes are suggested, then they will be discussed with supervisors and will be revised if necessary. Any changes to this document will be shared with all research members involved in the study.

Identification of Eligible Participants
This study utilises the Halo Medical Foundation's project area involving 35-40 villages of Osmanabad district. Each village has a village healthcare worker (VHW) and they work closely with pregnant women and adolescent girls in the village. There are two participant groups in this study 1) Pregnant women and 2) Adolescent girls.

The project area is divided into two blocks namely Tuljapur and Lohara. VHWs from each block meet once a month at HMF office. The primary investigator and data entry assistant will participate in these meetings, in support with VHWs and they will coordinate the study.

Pregnant women: Any woman who is a resident of the villages within the project area and between 3rd and 5th month of pregnancy is eligible to participate (onset of 3rd month of pregnancy and on or before completion of 5th month of pregnancy). VHWs conduct monthly meeting at village level involving pregnant women to educate them about antenatal care. The VHW maintains a list of all newly identified pregnant women in the village, which is updated through home visits every month if new pregnant women are identified. This study will utilise the VHW network of HMF and eligible pregnant women will be identified in coordination with VHWs in each village. The project will employ a data assistant who will work with the primary investigator. The assistant will work closely with VHWs to prepare an initial list of eligible pregnant women and the list will be updated on monthly basis.

Adolescent girls: We aim to collect data from 1000 adolescent girls from the study area. To do this, all villages will be ranked with an ascending order based on their population and recruitment will be from the smallest villages first. Any adolescent girl who is a resident of villages within the project area and between 13-17 years of age is eligible to participate. VHWs conduct a monthly discussion for adolescent girls at the village level and discuss issues about adolescent health and also provide health education materials. VHWs will prepare a list of adolescent girls between 13-17 years of age from each village and that will be used during the study to identify and to communicate with eligible participants. Data assistants will verify the list wherever necessary.

Data Collection
On identification of pregnant women or adolescent girls, data will be collected for each participant at village level with the support of the VHW. Data collection will take place either at VHW's house or participant's house or a community space (school/health centre) based on the convenience of each participant. The primary investigator will plan the data collection visits in coordination with data entry assistants and VHWs. If any of the eligible participant (from pregnant women group) is visiting the HMF's hospital for regular antenatal check-ups/out patient department then the participant will be approached at the hospital and will be informed about the study. In this case, the data collection and blood withdrawal will be

performed at the HMF's hospital. The blood tests namely complete blood count and glucose test will be performed free of cost for the participant with the support from HMF's hospital.

Recording Data

The study will use lists of eligible pregnant women and the girls from each participating village as outlined previously. Based on these lists, participants will be contacted by the VHW during a field visit. Participants who provide consent will be entered in the study. Those who do not consent will be recorded in a separate register; firstly to ensure that they are not approached again, and secondly to estimate the response rate, which will be maintained at the office by the primary investigator. Based on these registers, eligible lists and collected data, the response rate will be calculated. The register will have the names and villages of eligible participants who declined to participate in the study. There will be two individual registers at the office one for pregnant women and one for adolescent girls.

Medical Equipment

Medical equipment such as weighing scales, measuring tape, height measuring scales and blood pressure units are available in a standard calibrated form and do not need any manual adjustment. The study will use digital weighing machines and automatic blood pressure measuring units available by standard companies (opinion from HMF hospital staff will be considered during these purchases). Measuring tape for mid arm circumference and height measuring scale will be used by the data assistant/nurse/investigator. All equipment used in this research will be sound. Medical equipment will be checked by the primary investigator every month to ensure the accuracy (1st working day of each month) and the equipment performance report will be used to record this process. The verification will be performed with the equipment at the office, which means readings produced by an automatic blood pressure unit and weighing machine will be checked with the readings of a same person produced by office equipment. Project staff and other HMF staff will be requested to volunteer for this verification on monthly basis. The primary investigator will prepare a report of this verification every month and all equipment will be checked for accuracy on monthly basis. Similarly, if there is any wear and tear of measuring tape or height measuring scale, then the same will be replaced by a new unit. To ensure accuracy, the data entry assistant/nurse will be trained by the investigator to undertake readings for height, weight, blood pressure and mid arm circumference. In this study the mid arm circumference of the dominant hand will be taken and blood pressure of right hand will be taken for all participant. If any handicapped participant is involved, the blood pressure of left hand will be recorded and will be noted accordingly in the data collection form.

Blood Withdrawal

All blood withdrawals will be performed in a safe environment to ensure the safety of the participant as well as that of the technician. The environment will have sufficient light, and will not have any risk factors nearby such as fire, water, sharp objects, kitchen goods or utility tools. Participant will be allowed to leave after five minutes on completion of blood withdrawal to ensure that participant do not have any complaints such as blood oozing, pain and giddiness. The data entry operator and technician will ensure that participant is in comfortable position and safe environment during blood withdrawal. All haemoglobin readings will be noted in g/dl unit (example: 9.5 g/dl). All members involved in the study (nurse, technician, data assistant, VHWs) will receive training about the research conduct and the application of standard procedure at field level by primary investigator, which will be documented.

Pregnant women: For pregnant women a venous blood sample will be taken from participant's non-dominant hand with strict follow up of an aseptic protocol and the blood will be transferred into two tubes one will be EDTA/anticoagulant tube for complete blood count measurement. The second tube will be of fluoride solution for glucose tests. These tubes will be purchased from standard recognised companies and will be used in accordance with the protocol. Blood tubes will be transported in a secured recommended box to the HMF's hospital, where lab test will be performed by using cell counter machine for complete blood count and biochemistry analyser for glucose tests (Ice box will be used to carry blood tubes for glucose tests).

Adolescent girls: A finger prick method will be conducted for the Sahli's hemometer investigation to measure haemoglobin for adolescent girls. In this research, ring finger of non-dominant hand will be used for haemoglobin tests. This investigation will be performed at the village level by a qualified laboratory technician. Haemoglobin readings will be noted on data collection forms. Haemoglobin report will be shared with participant at the same time. There will not be any transport of blood samples in this group.

Subsequently, haemoglobin will be measured with non-invasive machine and will be recorded in data collection forms (NBM 200 machine). During the field visit, HMF nurse or lab technician will be accompanied with basic emergency medicines and will be trained to handle with emergencies. HMF's hospital will provide support during any emergency such as ambulances, emergency care and hospital admission. In such events all healthcare will be provided at no cost by HMF hospital.

Entry of Results and Communication

Pregnant women: The blood tests will be analysed at HMF's hospital and the report will be shared with participants over a telephone/through VHWs wherever appropriate. A copy of the report will be attached to the data collection form for data analysis and another copy will given to respective participants by VHW at village level. This involves reports of the complete blood count and glucose tests. Blood analysis will be completed within first 4 hours from withdrawal and reports will be prepared within 24 hours of withdrawal. Participants will receive information about their report within 24 hours (over telephone) and will receive paper copies of report within a week from test date by the VHW. However, any abnormal results in the pregnant women group (increase in glucose levels, severe anaemia, low platelet count, low neutrophils) then it will be informed to the primary investigator directly by lab technician and tests reports will be made available to the doctors, primary investigator and participants in first 24 hours. Hence the reports will be shared on urgent basis with participants and the referral process will be accelerated. The communication for urgent referrals between the hospital team, primary investigator and the study participant will be logged in a separate register. In case of absence of a primary investigator at the office, HMF's hospital doctor and lab technician will communicate with the participant. Primary investigator will be available on phone in case of emergency referral. Hb estimates obtained from Sysmex XP-100 will be used to identify anaemia status and based on the same clinical advice will be provided.

In the case of adolescent girls, haemoglobin results will be shared in village immediately after tests itself by lab technician/nurse/data entry assistant. Each adolescent girl will receive a health card with height, weight, haemoglobin record and advice for further referral. This health card will also contain information about anaemia and nutrition advice leaflet. Sahli's hemometer reading will be used for adolescent girls for any referral/clinical decision.

Referral Process

Pregnant women referral: Referral will be advised by the study investigator with support from doctors based at HMF's hospital, whenever necessary. Reports arising from the cell counter test and glucose estimation will be analysed by the doctors at the hospital, and accordingly the requirement of referral will be assessed along with the investigator. Similarly the final average of blood pressure will be analysed by the study investigator and those with blood pressure ≥140-90 mm Hg will be considered for medical referral. Primary investigator will hold records of the referral of pregnant women who have been diagnosed with severe anaemia and hypertension in a separate register.

Table 1: Haemoglobin reading and referral for pregnant women (National Rural Health Mission, Government of India)

No	Haemoglobin reading	Level of anaemia	Referral
1	11.0 g/dl or above	Not anaemic	No need of referral
2	10.0 to 10.9 g/dl	Mild anaemia	Refer to the Accredited Social Health Activist (ASHA)/ HMF's VHW/Government Nurse, based on health services availability in the village. Participants will receive blood tests reports, a report card and a nutrition advice leaflet.
3	7. 0 to 9.9 g/dl	Moderate anaemia	If haemoglobin value is from 8.1 to 9.9 g/dl Refer to the Accredited Social Health Activist (ASHA)/ HMF's VHW/Government Nurse, based on health services availability in the village. Participants will receive blood tests reports, a report card and a nutrition advice leaflet.
			If haemoglobin value is from 7.0 to 8.0 g/dl Participant needs medical consultation and services at secondary/tertiary care hospital. On priority basis, participant will be communicated by the primary investigator or doctors at the hospital and will discuss about the participant's preference (HMF's hospital/ Government hospital/Other private hospital) and will be referred for an urgent consultation. Additionally, participants will receive blood tests reports, a report card and a nutrition advice leaflet.
4	< 7.0 g/dl (6.9 g/dl and below)	Severe anaemia	Participant needs medical consultation and services at secondary/tertiary care hospital.

			On priority basis, participant will be communicated by the primary investigator or doctors at the hospital and will discuss about the participant's preference (HMF's hospital/ Government hospital/Other private hospital) and will be referred for an urgent consultation. Additionally, participants will receive blood tests reports, a report card and a nutrition advice leaflet.

All study participants from the pregnant women group will receive blood tests reports, a report card and a standardised nutrition advice leaflet and referral will be based on haemoglobin levels as outlined in the table. The nutrition leaflet will have information on iron rich food items, essential nutritious intake during the pregnancy and health services available by the HMF.

Table 2: Referral of diabetic participants from pregnant women group (World Health Organisation)

No	Timing of measurement		Referral
1	Random plasma glucose reading	≥ 200 mg/dl	If result shows random glucose level ≥ 200 mg/dl, then primary investigator will communicate with participant and they will be immediately referred to the HMF's hospital/ Government hospital/Other private hospital (considering participant's reference).

We will apply the outlined criteria to identify diabetes. The primary investigator will keep a separate set of record of pregnant women who have been diagnosed with diabetes during this research.

Hypertension referral: These participants will be called immediately for medical consultation at HMF's hospital where they will be seen by a doctor and will receive advice for the same. In case participant requests for a government hospital or another private hospital, then they will be referred accordingly. The study investigator will provide a referral note for participant's convenience. After the data entry in computer system, the primary investigator will verify hypertensive participants against the list of referred participants, which will provide an additional check. The study investigator will maintain a separate register containing the list of participants, who have been diagnosed with hypertension during thy study.

Table 3: Referral of Hypertensive Participants (World Health Organisation)

Participants with a final blood pressure ≥140-90 mm Hg will be identified as hypertensive. All identified hypertensive participant will be advised for medical consultation on priority basis and will be noted in a separate record as outlined above. Referral: Participant will be referred to the HMF's hospital/ Government hospital/private

hospital (considering participant's reference)

Pregnant women with platelet count < 150 X 10^3/ micro-litre will be labeled as thrombocytopenia. However during pregnancy physiologic thrombocytopenia (platelet < 100 X 10^3/micro-litre) is also observed especially in the third trimester and therefore the moderate and sever thrombocytopenia will be referred according the following protocol. The international guidelines by the American Journal Haematology suggests that the platelet count < 150 X 10^9/L is to be diagnosed with thrombocytopenia, therefore the value for micro-litre calculation will be a platelet value < 150 X 10^3/ micro-litre, and this will be used to interpret results. The available machine at the HMF's hospital will provide readings in micro-litre format. This is in accordance with the guidelines provided the manufacturer of the cell counter at the hospital.

Table 4: Referral of pregnant women with thrombocytopenia (American Haematology Association)

No	**Platelet reading**	**Level of thrombocytopenia**	**Referral**
1	100-150 X 10^3/ micro-litre	Mild	Participant will be informed about the mild thrombocytopenia and will be also advised for a routine follow up during their antenatal visits and if required then the facilities at HMF laboratory will be made available.
2	50-100 X 10^3/ micro-litre	Moderate	Participant will be referred to the HMF hospital/District government hospital at Osmanabad/ tertiary hospital at Sholapur considering participant's preference.
3	< 50 X 10^3/ micro-litre	Severe	Participant will be urgently referred within first 24 hours of tests results to the HMF hospital/District hospital at Osmanabad/ tertiary hospital at Sholapur considering participant's preference.

Pregnant women with neutrophil count < 2 X 10^3/micro-litre will be identified as neutropenia. Participants will be referred according the following protocol.

Table 5: Referral of pregnant women with neutropenia (NHS, UK)

No	**Neutrophil count**	**Referral**
1	< 2 X 10^3/ micro-litre	Participant will be informed about the mild neutropenia and will be also advised for a routine follow up during their antenatal visits and if required then the facilities at HMF laboratory will be made available.
2	< 1 X 10^3/ micro-litre	Participant will be referred to the HMF hospital/District government hospital at Osmanabad/ tertiary hospital at

		Sholapur considering participant's preference.
3	< 0.5 X 10^3/ micro-litre	Participant will be urgently referred within 24 hours of tests reports to the HMF hospital/District hospital at Osmanabad/ tertiary hospital at Sholapur considering participant's preference.

Referral of adolescent girls: Adolescent girls will receive a health card at village level, on completion of haemoglobin investigation. This card will have the participant's name, age, date of investigation, name of village, height, weight and haemoglobin reading. Further the card will have information about anaemia, haemoglobin tests and iron rich food items. This card will also provide information about accessing further help from government nurse, Accredited Social Health Activist (ASHA worker) or HMF's VHW. The card will also provide contact details of HMF's hospital where participant can access medical consultation. Primary investigator will hold records of referral of adolescent girls who have been diagnosed with severe anaemia in a separate register.

Table 6: Haemoglobin reading and referral for adolescent girls (National Rural Health Mission, Government of India)

No	**Haemoglobin reading**	**Level of anaemia**	**Referral**
1	12.0 g/dl or above	Not anaemic	No need of referral
2	11.0 - 11.9 g/dl	Mild anaemia	Refer to the Accredited Social Health Activist (ASHA)/ HMF's VHW/Government Nurse, based on health services availability in the village.
3	8. 0 - 10.9 g/dl	Moderate anaemi	If haemoglobin value is from 9.1 to 10.9 g/dl Refer to the Accredited Social Health Activist (ASHA)/ HMF's VHW/Government Nurse, based on health services availability in the village. If haemoglobin value is from 8.0 to 9.0 g/dl Participant will be communicated by the primary investigator or doctors at the hospital and will discuss about the participant's preference (HMF's hospital/ Government hospital/Other private hospital) and will be referred for a consultation with a doctor.
4	< 8.0 g/dl (7.9 g/dl and below)	Severe anaemia	Participant needs medical consultation and services at secondary/tertiary care hospital. On priority basis, participant will be

			communicated by the primary investigator or doctors at the hospital and will discuss about the participant's preference (HMF's hospital/ Government hospital/private hospital) and will be referred for an urgent consultation. Additionally, participants will receive a health report card and a nutrition advice leaflet.

The subsequent information in this document is developed for data assistant and other professionals involved in the research to ensure good conduct during the project implementation.

Data Security

All collected data from paper-based forms will be entered into the server computer by data assistant/computer operator, and this will be monitored by the primary investigator. On the verification of entered data the original data sheets will be stored in a filing cabinet, which will be accessible with a key only to the primary investigator. During post-delivery data collection for participated pregnant women, forms will be handled under the supervision of primary investigator or authorised permitted personnel. Data on the server computer will be anonymous and will not have any patient identification. The copy of the dataset with patient identification will be stored in a secured drive accessible only to the primary investigator. Anonymous data will be uploaded on Nottingham University drive (Research Drive) with the remote access service from India. This drive gives access to a folder named 'HaloData' accessible only to the members involved in this research. Data security will be ensured in this project by following the outlined protocol.

Basic Information

There are three types of data collection forms in this research.

1) Form for pregnant women: This form is indicated with capital **PW** mentioned on right corner on the front page of the form.
2) Form for adolescent girls: This form is indicated with capital **AG** mentioned on right corner on the front page of the form.
3) Form for village level data collection: This from is indicated with capital **VLD** mentioned on right corner on the front page of the form.

All three forms are available in both languages English and Marathi. Forms in Marathi language will be used for data collection. English version is available for office use only. Forms in Marathi language will have same indications outlined above as that of English version (**PW, AG** and **VLD** mentioned on the top right corner of the first page of each form).

Along with the data collection forms, participant information sheets are available for each study participant. There are three types of participant sheets one for each category.

(1) Participant information sheet for pregnant women indicated with **PW** on the top right corner.
(2) Participant information sheet for adolescent girls indicated with **AG** on the top right corner.
(3) Participant information sheet for HMF's village health worker and government nurse indicated with **VLD** on the top right corner.

Each participant should receive respective sheet, once they agree to participate in the study. Details of recruitment process and data collection process are mentioned further in this form.

List of Materials
Following material should be carried during data collection.

Non-medical equipment
1) Data collection form
2) Participant information sheet
3) Black ballpoint pen
4) Stationary includes pencil, stapler, scale, thumb ink pad (for those who cannot sign), notebooks and rain cover for your bag.
5) Standard operating procedure (this laminated sheet)

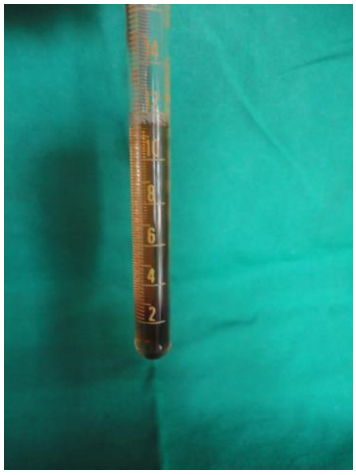
Medical equipment
1) Weighing machine
2) Measuring tape to measure mid arm circumference
3) Height measuring scale
4) Blood pressure measuring unit

The following units are used only by a qualified technician involved in this study.

5) Protection: protective gloves and eyewear for technician
6) Blood test for adolescent girls: Sterile disposable needles, Isopropyl alcohol swab, Haemoglobin measuring kit (Sahli's Hemometer), Surgical tape and Sterile Cotton.
7) Blood tests for pregnant women: Sterile disposable syringes, Arm tourniquet, Isopropyl alcohol swab, EDTA/anti-coagulant blood collection tube, Collection tubes for glucose (containing Fluoride solution), Sterile cotton, Surgical tape and Bandage.

Sahli's hemometer
This unit measures haemoglobin calorimetrically. It utilises the conversion of haemoglobin into acid haemtin, which is a brown colour solution. Sterile water is added to match this colour with comparators of the Sahli's unit. The match scale has standard readings illustrated at meniscus of the brown solution g/L unit, which will provide haemoglobin reading for the sample.





Good Practice

1) Always plan the field visit on communication with HMF's village health worker, primary investigator and lab technician.
2) Always carry the necessary materials including the standard operating procedure.
3) It's important to have your mobile phone with you in case you need to communicate urgently.
4) Use calculator provided with you for final average blood pressure calculations.
5) Blood withdrawal is permitted by authorized person only (lab technician or HMF's nurse).
6) Adolescent girls between 13 -17 years are only eligible to participate.
7) Pregnant women between 3 to 5 months of pregnancy are eligible to participate (at the start of 3 months until end of 5 month).
8) Provide sufficient time to participant to make the decision.
9) First obtain written consent then proceed with data collection
10) Do not use any other ink or other pen than the one provided in your kit.
11) Do not overwrite or do not write outside any boxes provided on data collection form.
12) Use extra white sheets provided in your kit, if required. Please staple these sheets to the main data collection form.
13) Maintain record of each visit at office register.
14) Complete field visit report for each day of work.
15) Weighing machine and automatic blood pressure units are battery operated and carry extra batteries during fieldwork.
16) It is mandatory to store your material, data collection forms, field visit bags and other records in allotted space at office only.
17) Please discuss with primary investigator to clarify any doubts.
18) Use operating procedures given in Marathi language if necessary.

Step by step guide for data collection process at field level

A) Standard Guide of data collection- The following sequence is a standard process of data collection and is mandatory to follow each time by person involved in this study.

Plan your visit and communicate with
(1) Primary investigator (2) HMF's village health worker (3) Medical staff lab technician/HMF's nurse (4) Vehicle driver

↓

Data collection needs to be completed at village level in village health worker's house or participant's house, considering participant's preference.
Call only one eligible participant at a time for an interview (one pregnant women or one adolescent girl)

→ If eligible participant is available on another day, then note in your diary and confirm the apportionment

↓

Provide complete information about the project and request for participation. Provide sufficient time to make a decision. It is must to have consent from parent/local guardian for all adolescent girls.

→ We need a list of all eligible participants who declined to involve in this study.

↓

If participant is eligible but declined to participate in this study then please note on data collection form (second page) and use a new form for another participant.

↓

Once participant agrees to participate, then first take the written consent on the second page with all details mentioned along with signature/thumb impression of witness present at the time of data collection.

↓

- Follow the sequence of data collection from
- There is a guide on subsequent page for few question, check those if you have any doubt.
- If still doubt persist then note on your writing pad, and communicate (via phone) with primary investigator at the end of session.
- Give sufficient time to participant to understand each question.
- Repeat the question if required.
- Request to repeat the answer if you have not interpreted accurately.
- Each participant will go through blood tests and after ensuring all data is entered, visit new participant.

B) Pregnant Women data collection guide- The following sequence will help to complete pregnancy related data collection. Page numbers are mentioned according to the English version and Marathi as well so assess clearly.

Page 1: On right corner you can see **PW** indicating that this form should be used for pregnant women. First page provides information about this research project, participant rights and contact details. These details are similar to those on participant information sheet for pregnant women. If participant is unable to read this information in Marathi then explain them about purpose of the project, study area, participant rights, benefits with participation, investigation involved in the study and how much time you require to collect this data. Provide sufficient time to each participant to make a decision. You are not supposed to write on this page.

Page 2: Make sure that participant has understood all eight points on the page 2 and then proceed for consent. Please write the name of participant in the space provided in section 9) on this page. Take written consent from participant, complete contact details, address, witness signatures and then mention your own details (name, date and sign). Write a date of data collection clearly. Space allotted for primary investigator will be used later by Anand. Please ensure that all information for written consent is obtained first, and later you proceed to page 3. If primary investigator is collecting the data then write 'NOT INVOLVED' in data entry assistant section.

Page 3,4: You can use abbreviations as noted in each box and follow the sequence. Please note height, weight, mid arm circumference by using standard equipment provided with you. There are instructions for Blood pressure readings in Row 12 and as well as they are outlined below. Please see example for further assistance. Row 13 is for the use of primary investigator; do not write in this row (13).

- Use appropriate cuff considering participant requirement.
- Two-minute interval between consecutive readings.
- Take minimum of two readings. If the difference between first two readings of systolic blood pressure is more than 5 mm of Hg, then take further readings until the difference of two consecutive readings is ≤ 5 mm of Hg.
- Use the average of these most recent two readings to generate the final blood pressure.
- If more than five readings are required then use space provided at the end of page 6 of PW form (English version) and page 9 in Marathi version.

Example: See the detailed explanation below.

Reading count	**Systolic Blood pressure**	**Diastolic Blood Pressure**
First reading	145	85
After first reading, now take one more after 2 minutes of break.		
Second reading	133	88
See the difference between first two systolic blood pressure readings- It is 12 which is more than 5. (145-133 = 12). Hence we take further a third reading.		
Third reading	126	85
See the difference between the (most recent reading i.e.) second and third systolic blood pressure is 6, which is more than 5. (133-126 = 6). Hence we take further fourth reading.		
Fourth reading	122	85
See the difference between the (most recent reading i.e.) **third and fourth systolic blood pressure is <u>4, which is less than 5.</u> Hence we can now stop recording blood pressure and take average of most two recent reading.**		
Fifth reading	We do not require fifth reading as difference between the most recent two readings is less than 5, as shown above.	
Final average reading (of most two recent reading)	126+122= 248, 248/2 = 124 So the blood systolic pressure is 124 **Note only <u>124</u>, in this box, do calculation on your own notes book**	85

Measurement of Blood Pressure

1) Automatic blood pressure unit will be used in this study. This is how your automatic blood pressure unit looks like. You have to select appropriate handcuff. There are two sizes, and make sure that the one you use fits properly and intact on participant's upper arm while taking readings.



2) Please ask participant to use a chair and the hand should be supported on a table or a flat surface as shown in the picture below. If participant is in sitting position on floor then please use stool or a box to support the hand. It is important that the hand should be resting on a straight surface.



3) The cuff of this unit needs to be adjusted properly around the upper arm of participant. The cuff should be intact and tight. Keep the tubing of the blood pressure straight and upwards as shown in the following picture.
4) Make sure that screen of blood pressure unit is on and connections are proper before you press **START** button for taking readings as shown below.



5) Enter readings from the screen on data entry sheet. Follow blood pressure reading criteria as explained previously.

Measuring Height

1) Use the standard height measuring scale provided in your kit.
2) Participant needs to stand close to a wall or across a flat surface as shown in pictures below.





3) Make sure that feet of participant are flat and then adjust the scale over participant's head as shown in the picture below. Use wooden/metal stool if you need to adjust the scale accurately.
4) Hold the scale at position firmly and ask participant to leave and accurate reading will be visible.
5) Note readings in allotted space on data collection form.

Measuring Weight

1) Use the standard digital weighing machine provided in your kit.





2) Participant needs to stand straight on the machine and should facing straight.
3) Make sure that the digital screen of machine is visible, ask participant not to move and once readings on the screen is stabilised note in allotted space on data collection form.
4) The kilogram sign (KG) will appear on the screen, once reading is stabilised. Note down accurate weight on form.

Measuring mid arm circumference

1) You will need a measuring tape from your equipment kit.
2) Mid arm is a midpoint of upper arm length between shoulder and elbow, as shown in the following picture.





3) You can see in the first picture midpoint is denoted with scale. You can assess mid-point from sideways clearly.
4) Once you confirm the mid-point, use your tape to circle the arm at the midpoint place, as shown in the picture below. Hold initial 0 on tape and then circle back to the zero with tape to see reading.
5) Please hold the measuring tape tight and intact in a straight line as shown above.
6) Ask VHW to help if participant is more comfortable with female taking measures, if so then please ask assistance from VHW.

Page 4: Note information about medication carefully. Questions like 23, 24 on the page 4 (English version), Page 6 (Marathi version) are sensitive, thus provide sufficient time to participant. Use space allotted after the question 25, if participant has more than 3 boys and 3 girls. Write by mentioning B4:___Years or G4:___Years. Circle only one option for the question 26.

For the question 28 about house structure, read following if you require any assistance. Details should be about the house, which belongs to the participant or her husband.

Temporary house defined as a structure made up with animal dung/wood/ and has a roof made up of metal or wooden sheets.

- Semi-permanent house is defined as a structure made up with the use of cement, bricks/stones but has NO concrete roof (NO cement slab).
- Permanent house is defined as a structure made up with the use of cement, bricks/stones and has a concrete roof (cement slab).

Page 5, 6: Questions from 27 to 38 are about the participant and her husband's assets only (immediate family). Please make sure that participant understands this. Space at the end of page six is for blood pressure readings; if the previous space on the page 3-4 was not adequate then use this section to note additional blood pressure readings. Blood withdrawal will take place

once you complete all information. Further pages 7, 8 are for primary investigator. In Marathi form, you can use space provided on 9 to write blood pressure readings.

- Verify all information is entered from Page 1 to Page 6 (English), while unlit Page 10 (Marathi).
- Provide participation information sheet.
- Provide health report card.

End of data collection form PW.

C) Adolescent Girl data collection guide- The following sequence will help to complete data collection with adolescent girl groups.

Page 1: On right corner you can see **AG** indicating that this form should be used for adolescent girl. First page provides information about this research project, participant rights and contact details. These details are similar to those on participant information sheet for adolescent girl. If participant is unable to read this information in Marathi, then please explain them about the project, highlighting the purpose of this research, study area, participant rights, benefits from participation, investigation involved in the study and how much time you require to collect data. Provide sufficient time to each participant to make a decision. You are not supposed to write on this page.

Page 2: Make sure that participants have understood all eight points on the page 2 and then proceed to consent section. Please write the name of participant in the space provided in section 9) on this page. Take written consent from participant, complete contact details, address and take written consent from participant's parent (father/mother/grandparent) or a local guardian. This is very important as all adolescent girls in the study are between 13-17 years old. Then mention your own details (name, date and sign). Write a date of data collection. The subsequent space allotted for primary investigator will be used later by Dr Anand Ahankari. Please ensure that all information for written consent is obtained and then proceed to page 3. If primary investigator is collecting the data then leave data entry assistant section blank.

Page 3: Follow the sequence to complete the form, Use abbreviation as mentioned in the form. Please note height, weight, mid arm circumference by using standard equipment provided with you. See previous pictures if required.

Page 4,5: For question 17, 18 select only one option.

Use the following information for question 18 about the house structure.
Details should be about the house, which belongs to the participant's parents. Make sure the participant understands this clearly.

- Temporary house defined as a structure made up with animal dung/wood/ and has a roof made up of metal or wooden sheets.
- Semi-permanent house is defined as a structure made up with the use of cement, bricks/stones but has NO concrete roof (NO cement slab).
- Permanent house is defined as a structure made up with the use of cement, bricks/stones and has a concrete roof (cement slab).

Page 5, 6: There are question about menstrual periods (question number 30,31,32,33) and if participant is not comfortable or not willing to answer then please mention D (declined to answer), in the space allotted. If you need help with these questions, involve VHW in the interview. Take haemoglobin readings and note in the space (ringer finger of non-dominant hand). Write first name of person who has done haemoglobin tests in the space provided.

Health Report Card: Provide a health card to each participant mentioning height, weight, haemoglobin record, date of tests/data collection and TICK appropriate referral option. Make sure that you have entered all information on this card.

- Verify all information is entered from Page 1 to Page 5 (English version) and until Page 6 (Marathi version).
- Provide participation information sheet.
- Provide health report card.

End of data collection form

D) Village level data

This data will be collected once during the study period involving HMF's VHW and government nurse (if available) through an interview for 10-15 minutes at village level or at the HMF's office. All data will be collected from the participating villages in the same two months of period to ensure standardized data that permits comparison across villages. There will not be any investigation in this section. Participant will receive information sheet about the project. The data will be stored on a computer and will follow security protocol as outlined previously in this document.

Office will hold following records and will be maintained by the primary investigator.

Office Registers

1) Staff attendance
2) Village list and VHW contacts
3) Eligible list of pregnant women
4) Eligible list of adolescent girls
5) Decline to participate in the study: Pregnant women
6) Decline to participate in the study: Adolescent girls
7) Participated in the study: Pregnant women
8) Participated in the study: Adolescent girls
9) Pregnant women with diagnosed hypertension
10) Pregnant women diagnosed with diabetes
11) Pregnant women with severe anaemia referral register
12) Pregnant women with sever thrombocytopenia and sever neutropenia referral register
13) Urgent referral communication register
14) Adolescent girls severe anaemia referral
15) Letterhead register (for patient's referral requests if asked and for primary investigator use)
16) Staff (VHWs and data assistant) meeting and training register
17) Stock register

Office Files

1) PhD application
2) Research protocol
3) Changes in Protocol
4) Reports to Ethics committee
5) Ethics application and approval
6) Meeting notes
7) Research form data collection forms (English and Marathi)
8) Data forms storage total 3, one for each
9) Referral Register

10) Grant application documents
11) Standard operating procedures (laminated sheets)
12) Translation file

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Mention 'ACP MAS-SOP 2014 Print Requests' in the subject line.

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Appendix 9: Individual level data variables selected for modelling the association between each covariate and anaemia

No	Information collected on the following variables	Type of variable	Included in the unadjusted analysis	Included in the adjusted analysis	If excluded from the adjusted analysis, then explain here
Adolescent girl dataset					
1	Age in years	Continuous	Yes	Yes	-
2	Current education status	Categorical	Yes	Yes	-
3	Temporary paid work	Categorical	Yes	No	This does not indicate constant guaranteed income, and may change with season/availability of farming work, thus current education status, and the overall socioeconomic status (using parental farming land) were selected in the adjusted model.
4	Handedness	Categorical	Yes	No	This variable was not directly related with anaemia.
5	At least one elder sibling	Categorical	Yes	Yes	-
6	At least one younger sibling	Categorical	Yes	Yes	-
7	Mid upper arm circumference (MUAC)	Categorical	Yes	Yes	-
8	Body Mass Index	Categorical	Yes	No	MUAC was selected as a more reliable nutrition measure over BMI.
9	Any diagnosed disease	Categorical	Yes	No	Only 5 participants had a disease (out of 1010), and those were not directly related with anaemia (included epilepsy and speech disorder).
10	Consumed iron folic acid supplement in the past	Categorical	Yes	No	The current IFA consumption was included.

11	Currently taking iron folic acid supplement	Categorical	Yes	Yes	-
12	Currently taking calcium supplements	Categorical	Yes	No	This variable was not directly related with anaemia.
13	Currently taking any other medication including multi-vitamins	Categorical	Yes	No	This variable was not directly related with anaemia.
14	Religion and category (open/reserve)	Categorical	Yes	Yes	-
15	House structure	Categorical	Yes	No	Only one variable was selected to measure socioeconomic status (parental farming land).
16	Parents possess farming land	Categorical	Yes	Yes	-
17	Parents possess livestock	Categorical	Yes	No	Only one variable was selected to measure socioeconomic status (parental farming land). Livestock may change as it is easy to sale animals in our field setting.
18	Parents possess television set	Categorical	Yes	No	Only one variable was selected to measure socioeconomic status (parental farming land).
19	Parents possess mobile phone	Categorical	Yes	No	As explained above
20	Registered under the below poverty line families (BPL)	Categorical	Yes	No	Published research (including a study from MAS) showed that BPL allocation in India had number of issues mainly related with its eligibility, thus parental farming land was a reliable measure of socioeconomic status in our population.
21	Dietary preference	Categorical	Yes	No	Individual dietary variables providing specific diet information were selected instead of

					this single categorical variable.
22	Diet recall Pure milk	Categorical	Yes	Yes	-
23	Green leafy vegetables	Categorical	Yes	Yes	-
24	Bean sprouts	Categorical	Yes	Yes	-
25	Pulses-lentils	Categorical	Yes	Yes	-
26	Fruits/Fruit juices	Categorical	Yes	Yes	-
27	Rice	Categorical	Yes	Yes	-
28	Eggs	Categorical	Yes	Yes	-
29	Chicken	Categorical	Yes	Yes	-
30	Goat meat	Categorical	Yes	Yes	-
31	Heard about anaemia and haemoglobin	Categorical	Yes	No	These three variables (31, 32, 33) had discrepancies, which are due to recall biases, thus were not included in the model.
32	Tested haemoglobin before	Categorical	Yes	No	As explained above
33	Aware about your recent haemoglobin levels	Categorical	Yes	No	As explained above
34	Attained menarche	Categorical	Yes	Yes	-
35	Know the age at menarche	Continuous	Yes	No	The issue of recall bias was observed and thus these two variables (35,36) were not included in the model. While the variable 34 (attained menarche) was observed as a reliable measure, and many participants reported the exact date of the start of their first menstrual period, thus included in the model.
36	Missed school due to menstrual cycle	Categorical	Yes	No	As explained above
Pregnant women dataset					
1	Current pregnancy month	Categorical	Yes	No	The study collected information only once in pregnancy from 3 to 5

					months of pregnant women, thus pregnancy month had limited role in the analysis.
2	Current age	Continuous	Yes	Yes	-
3	Consanguineous marriage	Categorical	Yes	Yes	-
4	Age at marriage	Categorical	Yes	No	The legal age for marriage is 18 years in India, and all efforts were made to avoid any reporting bias in the study. This variable had a limited role, thus the current age of participant was included in the model to investigate risks of anaemia.
5	Education	Categorical	Yes	Yes	-
6	Last menstrual period known (LMP)	Categorical	Yes	No	This variable was not directly related with anaemia.
7	Body Mass Index (BMI)	Categorical	Yes	No	MUAC was selected as a reliable nutrition measure over BMI.
8	Mid upper arm circumference (MUAC)	Categorical	Yes	Yes	-
9	Diagnosed with any diseases	Categorical	Yes	No	None of the participants had any diagnosed diseases.
10	Currently taking iron folic acid supplements (IFA	Categorical	Yes	Yes	-
11	Currently taking calcium supplements	Categorical	Yes	No	This variable was not directly related with anaemia.
12	Currently taking multivitamins	Categorical	Yes	No	This variable was not directly related with anaemia.
13	Current gravidity	Continuous	Yes	Yes	-
14	Total live births	Categorical	Yes	No	The current gravidity was included in the model, which was a reliable measure than total live births.
15	Previous stillbirths	Categorical	Yes	No	This variable was not directly related with anaemia. Those who experienced stillbirths

					were less than 1%; thus had a limited role in the analysis.
16	Religion and category	Categorical	Yes	Yes	-
17	Annual income (in INR)	Categorical	Yes	Yes	-
18	House structure	Categorical	Yes	No	Only one variable to measure socioeconomic status was selected in the model. Information on family income was collected, and found to be a reliable measure than this variable.
19	Farming land (in acres)	Categorical	Yes	No	As explained above
20	Possess motor pump at farm	Categorical	Yes	No	As explained above
21	Possess motorbike	Categorical	Yes	No	As explained above
22	Possess car/vehicle	Categorical	Yes	No	As explained above
23	Possess television	Categorical	Yes	No	As explained above
24	Possess mobile	Categorical	Yes	No	As explained above
25	Possess livestock	Categorical	Yes	No	As explained above
26	Registered under below poverty families (BPL)	Categorical	Yes	No	Published research (including a study from MAS) showed that BPL allocation in India had number of issues mainly related with its eligibility, thus family income was a reliable measure of socioeconomic status in our study population.
27	Sold any assets in past 12 months	Categorical	Yes	No	Only one variable to measure socioeconomic status was selected in the model. Information on family income was collected, and found to be a reliable measure than this variable.
28	Dietary preferences	Categorical	Yes	No	Individual dietary variables providing specific diet information were selected instead of this single categorical variable.
29	Dietary recall				

	Pure Milk	Categorical	Yes	Yes	-
30	Green leafy vegetables	Categorical	Yes	Yes	-
31	Bean sprouts	Categorical	Yes	Yes	-
32	Pulses-lentils	Categorical	Yes	Yes	-
33	Fruits/juices	Categorical	Yes	Yes	-
34	Rice	Categorical	Yes	Yes	-
35	Eggs	Categorical	Yes	Yes	-
36	Chicken	Categorical	Yes	Yes	-
37	Goat meat	Categorical	Yes	Yes	-
38	Cooking fuel	Categorical	Yes	No	This variable was not directly related with anaemia.
39	Heard about anaemia and Haemoglobin	Categorical	Yes	No	These three variables (39, 40, 41) had discrepancies, which are predominantly due to recall biases, thus were not included in the model.
40	Checked Hb during current pregnancy	Categorical	Yes	No	As explained above
41	Know recent Hb levels	Categorical	Yes	No	As explained above
42	Pregnancy registration	Categorical	Yes	Yes	-
43	Maternal anaemia	Categorical	Yes	No	Maternal anaemia was the primary outcome in the pre-delivery analysis. In the post-delivery analysis, maternal anaemia thought to be an effect modifier on the casual pathway of consanguineous marriage and low birth weight, thus excluded in the fully adjusted model. However, a sensitivity analysis was conducted by including maternal anaemia and the same is reported in the thesis.

Appendix 10: Village level data variable selection for modelling

The following table illustrates decisions made on village level factor selection and analysis. Necessary consultation was facilitated with HMF team and also with PhD supervisors during the process.

No	Information collected on the following variables	Type of variable	If all 34 villages are in one category (such as 34 in yes OR 34 in no), then mention No Regression conducted (NR)	Is it relevant to adolescent health? Included/excluded	Is it relevant to pregnancy health? Included/excluded
1	Village level governance body	Categorical (no/yes)		EXCLUDED, none of the programmes/initiatives of governance body are related with anaemia	EXCLUDED, none of the programmes/initiatives of governance body are related with anaemia
2	Presence of women members in village governance body	Categorical (no/yes)		As noted above	As noted above
3	Presence of village health sanitation committee	Categorical (no/yes)		EXCLUDED, 30 villages had the committee, but less than 5 are operational. No budget for adolescent health, the committee has no mandate on anaemia	EXCLUDED, 30 villages had the committee, but less than 5 are operational. The committee has no mandate on anaemia
4	Presence of village education committee	Categorical (no/yes)	NR	Omitted	Omitted
5	Public phone availability	Categorical (no/yes)		EXCLUDED, not related with health status, 93% participants had mobile	EXCLUDED, not related with health status, 90%

				phone at home. Only 7 villages had public phone.	participants had mobile phone at home
6	Water purification facility	Categorical (no/yes)	NR	Omitted	Omitted
7	Piped water supplied to houses	Categorical (no/yes)		Included, important resource, young girls support their mother to store water so continuous water supply may improve their health by preventing dehydration caused mainly in summer/ or overall health may improve by preventing physical exertion	Included, important resource, women are likely to invest less time to bring water if its directly supplied to their house, may improve health status
8	Water tanks/hand pumps availability	Categorical (no/yes)		EXCLUDED, in many villages hand pumps/water tanks are not operational, no additional data to verify any post analysis claims	EXCLUDED, many villages hand pumps/water tanks are not operational, no additional data to verify any post analysis claims
9	Daily water supply (at least once a day)	Categorical (no/yes)		Included, important resource	Included, important resource
10	Operational community toilets	Categorical (no/yes)		Included, important from hygiene perspective	Included, important from hygiene perspective
11	Alcohol sold in village	Categorical (no/yes)		EXCLUDED, not related to adolescent anaemia	EXCLUDED, not related to pregnancy anaemia
12	Government health centre availability (sub-centre/primary health	Categorical (no/yes)		Included, health access	Included, health access

	centre)				
13	Accredited social health attendant (ASHA) appointed	Categorical (no/yes)		Included, important to access medicines (includes IFA)	Included, important to access medicines (includes IFA)
14	Government nurse appointed	Categorical (no/yes)	NR	Omitted	Omitted
15	Daily access to government nurse	Categorical (no/yes)		Included, health access	Included, health access
16	Government nurse provides antenatal care services	Categorical (no/yes)		EXCLUDED, not related to adolescent anaemia	Included, health access in pregnancy
17	Government nurse provides medicines	Categorical (no/yes)	NR	Omitted	Omitted
18	Government nurse conducts haemoglobin investigation	Categorical (no/yes)		Included, primary health access in the study area	Included, primary health access in the study area
19	Blood pressure check-up facility	Categorical (no/yes)		EXCLUDED, not related to anaemia	EXCLUDED, not related to anaemia
20	Private doctor availability	Categorical (no/yes)		Included, health access	Included, health access
21	Emergency health service availability	Categorical (no/yes)		EXCLUDED, not related to adolescent anaemia	EXCLUDED, not related to pregnancy anaemia
22	Government transport availability	Categorical (no/yes)		EXCLUDED, not related to adolescent anaemia	EXCLUDED, not related to pregnancy anaemia
23	Private transport availability	Categorical (no/yes)	NR	Omitted	Omitted
24	Nearest highway from village	Categorical (1/2)		EXCLUDED, not related to adolescent anaemia	EXCLUDED, not related to pregnancy anaemia
25	Distance to nearest	Continuous		EXCLUDED, not related to	EXCLUDED, not

	highway			adolescent anaemia	related to pregnancy anaemia
26	Travel time to the nearest primary care hospital	Continuous		Included, health access, better measure than other transport related variables outlined above	Included, health access, better measure than other transport related variables outlined above
27	Government ambulance availability	Categorical (no/yes)	NR	Omitted	Omitted
	Summary				
	Total number of factors proposed for inclusion in adolescent girl (chapter 3)	9			
	Total number of factors proposed for inclusion in pregnancy women (chapter 4)	10			

Appendix 11: Joinpoint regression programme

a. Background
This is a Microsoft Windows-based statistical software programme that was developed by the National Cancer Institute in the USA, as part of their cancer surveillance programme. The software allows the user to test whether or not an apparent change in a temporal trend is statistically significant. The Joinpoint software fits the selected data (example: Maharashtra Birth Registry Dataset used in this PhD Research) into the simplest Joinpoint model allowed by the data, and the user selects how many Joinpoint or inflexion points are allowed in the model. We only used one Joinpoint inflexion point for the analysis of the Maharashtra Birth Registry Dataset as there were only 32 points of data (one for each year) and we wanted to maximise the power of the analysis.

This regression technique is commonly used to investigate trends over a period of time (example cancer trends, sex ratio trend). This allows understanding benefits of any public health interventions, and its effects on a wider population level by analysing changes in disease trends/rates. It is relatively novel for statistical testing, as it is hypothesis free and driven by the data rather than a prior hypothesis. Hence, it is best used in conjunction with knowledge of the context of the data being tested, and ideally a prior hypothesis that can be used to interpret the output of the Joinpoint model (as in the MAS analysis we used the dates of Indian anti-foeticide legislation).

b. Technique
The method is similar to the minimisation of least squares regression, in which Joinpoint finds the best-fit line through several years of data. It uses the variance to tests whether a multi-segmented line is a significantly better fit than a straight or less segmented line. Line segments are joined at points called Joinpoints. Each point indicates a statistically significant change in trend ($p < 0.05$). The significance test uses a Monte Carlo Permutation method using which it finds the best-fit line in a given dataset. The graph generated using Joinpoint plots rate/ratio (on y axis) against years/time period (on x axis).

The results are generally presented using following parameters:

- Annual Percent Change, Average Annual Percent Change (indicated as APC and AAPC respectively)
- Joinpoint trend (could range from 1 to maximum permitted)
- Significance presented using p value (two sided)
- Joinpoint Graph presenting the best-fit line is presented.

c. Additional readings and resources (web links as per 4 September 2017)

- Additional information about the JoinPoint Regression Programme is available at: https://surveillance.cancer.gov/joinpoint/
- To read more about the method and applications of JoinPoint, use the following web link: https://surveillance.cancer.gov/help/joinpoint/statistical-notes/references

Appendix 12: List of publications

A. Journal Publications

From PhD dataset (7 papers, 2013-17, updated on 1st October 2017)

1. **Ahankari AS**, Myles PR, Dixit JV, Tata LJ, Fogarty AW. Risk factors for maternal anaemia and low birth weight in pregnant women living in rural India: a prospective cohort study. **Public Health** July 2017. doi: 10.1016/j.puhe.2017.06.023.

2. **Ahankari AS,** Fogarty AW, Tata LJ, Myles PR. Healthcare benefits linked with Below Poverty Line registration in India: Observations from Maharashtra Anaemia Study (MAS). **F1000Research** January 2017. doi: 10.12688/f1000research.10556.1

3. **Ahankari AS**, Myles PR, Tata LJ, Fogarty AW. PCPNDT seems to benefit India's birth sex ratios. **BMJ** September 2016**.** doi: 10.1136/bmj.i5194.

4. **Ahankari AS**, Myles PR, Fogarty AW, Dixit JV, Tata LJ. Prevalence of iron deficiency anaemia and risk factors in 1,010 adolescent girls from rural Maharashtra, India: a cross-sectional survey. **Public Health** August 2016. doi: 10.1016/j.puhe.2016.07.010.

5. **Ahankari AS**, Dixit JV, Fogarty AW, Tata LJ, Myles PR. Comparison of the NBM 200 non-invasive haemoglobin sensor with Sahli's hemometer among adolescent girls in rural India. **BMJ Innovations** August 2016. doi: 10.1136/bmjinnov-2015-000139.

6. **Ahankari AS,** Fogarty AW, Tata LJ, Dixit JV, Myles PR. Assessment of a non-invasive haemoglobin sensor NBM 200 among pregnant women in rural India. **BMJ Innovations** February 2016. doi: 10.1136/bmjinnov-2015-000085.

7. **Ahankari AS,** Myles PR, Tata LJ, Fogarty AW. Banning of fetal sex determination and changes in sex ratio in India. **Lancet Global Health** September 2015. doi: 10.1016/S2214-109X(15)00053-4.

Other manuscript publications (11 papers, 2013-17, updated on 1st October 2017)

1. **Ahankari AS,** Myles PR, Tsang S, Khan F et al. A qualitative study exploring factors influencing clinical decision-making for influenza-like illness in Solapur city, maharashtra, India. **Anthropology and Medicine** 2017. doi: 10.180/13648470.2017.1321459.

2. Adaji EM, **Ahankari AS**, Myles PR. An investigation to identify potential risk factors associated with common chronic diseases among the older population in India. **Indian J Community Medicine** 2017. doi:10.4103/0970-0218.199802

3. **Ahankari A,** Bapat S, Myles P, Fogarty A, Tata L. Factors associated with preterm delivery and low birth weight: a study from rural Maharashtra, India**. F1000Research** 2017. doi: 10.12688/f1000research.10659.1

4. Laur C, Ball L, **Ahankari AS**, Advagovska M et al. Proceedings of the Inaugural International Summit for Medical Nutrition Education and Research, Cambridge, UK. Public Health 2016. doi: 10.1016/j.puhe.2016.08.023.

5. Marufu T, **Ahankari A,** Coleman T, Lewis S. Prenatal maternal smoking and stillbirth: A systematic review and meta-analysis. **BMC Public Health 2015**. doi: 10.1186/s12889-015-1552-5.

6. **Ahankari A,** Marufu T. India's progress towards 5th Millennium Development Goal: A critical review. **Indian J Matern Child Health 2015**;16:1-11.

7. **Ahankari AS,** Leonardi-Bee J. Maternal hemoglobin and birth weight: A systematic review and meta-analysis. **Int J Med Sci Pub Health 2015**. doi**:** 10.5455/ijmsph.2015.2212201489.

8. **Ahankari AS,** Fogarty A. Association of Socioeconomic status with sex ratio at live birth in Individuals living in the slums of Sholapur city, India. **Int J Med Sci Pub Health 2015**. doi:10.5455/ijmsph.2015.1109201428.

9. Otete H, **Ahankari AS,** Jones H, Bolton K, Jordan C et al. Parameters for the mathematical modeling of clostridium difficile: A systematic Review. **PLoS ONE 2013**. doi: 10.1371/journal.pone.0084224.

10. **Ahankari AS**, Bhorgonde D. Navigational system in dental implants. **KSDJ 2012**; 31:32-2.

B. Published manuals/handbooks

1. Ahankari A (Eds). Life free from violence: training manual for village based dispute resolution committee members. Halo Medical Foundation, 3rd Edition. June 2014.
2. Dixit J. Ahankari A. Maternal and neonatal health training manual for grass root health workers. Halo Medical Foundation, India. April 2013.
3. Dixit J. Ahankari A. Adolescent health dialogue booklet. Halo Medical Foundation, India. April 2013.

C. Conferences

Conference presentations

1. Medical Education and Nutrition Training in India (Presentation, 1st Annual Summit of The Need for Nutrition Education/Innovation Programme, UK 2015).
2. Factors influencing the clinical diagnosis and management of patients presenting with influenza like illness in Sholapur, India (Poster, 14th World Congress on Public Health, India, 2015).
3. Prevention and intervention of domestic violence against women: A SAVALI Project (Poster, 19th International congress on women health, ICOWHI, Thailand, 2012).
4. Self-evaluation and satisfaction of dental aesthetic among various professional college students in Bidar city, Karnataka (Poster, International dental conference and symposium, Hong Kong, 2010)

Conference participation

1. Preterm birth: Immediate care (UK, 2016)
2. The Need for Nutrition Education/Innovation Programme, 2nd Annual summit (UK, 2016)
3. Women Deliver Congress (Denmark, 2016)
4. 3rd International student meet of Public Health (India, 2015).
5. The chief nursing officer's black and minority ethnic advisory group: 5th annual conference (UK, 2013).
6. Royal college of pediatrics and child health: annual congress (UK, 2012).
7. Tropical Epidemiology Group meeting, London School of Hygiene & Tropical Medicine (UK, 2012).
8. UK national smoking cessation conference (UK, 2012)