Cover letter
6th October 2017

Professor Sarah Lewis
Division of Epidemiology and Public Health
University of Nottingham, Nottingham

Subject: Submission of the revised PhD thesis

PhD student details, Name: Anand Ahankari, Student ID: 4200341

Dear Professor Lewis,

Thank you for examining my PhD thesis, and providing recommendations.

In this cover letter, I have explained changes made in the thesis with additional details such as page numbers where the changes are inserted in the thesis file.

Along with this cover letter the following two documents are submitted.

1. Revised thesis file with the track changes (*MS word file*).
2. Revised thesis free from track changes/clean version (*MS word file*).

Yours Sincerely,

Anand Ahankari
PhD student
Division of Epidemiology and Public Health
University of Nottingham, UK

Recommendation 1

Coherence: Ensure that various studies, and especially study 3 are brought well into a coherent entity. This includes revisiting the introduction, rationale, aims and objectives and possibility the final conclusion and recommendations. Its up to Anand and his supervisor to come up with an approach to make this revision.

Response: *To address this, I have made following changes in the introduction and methodology chapters.*

- *PhD background and rationale of the study are rewritten.*
- *Project goal is now revised to fit the study 3 with the other two anaemia studies.*
- *Aims and objectives from chapter 2 (Methods) are moved to chapter 1 (Introduction). This is followed by figure 2, which summarises objectives for each study.*
- *Table of contents, and figures are also updated accordingly.*
- *Changes in the recommendations are provided at the end of this cover letter.*

Changes (page numbers: 7 to 10 and 16 to 22). The revised section from the introduction chapter is outlined below for your kind reference.

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.

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.

Goal and objectives

Study goal

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

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.

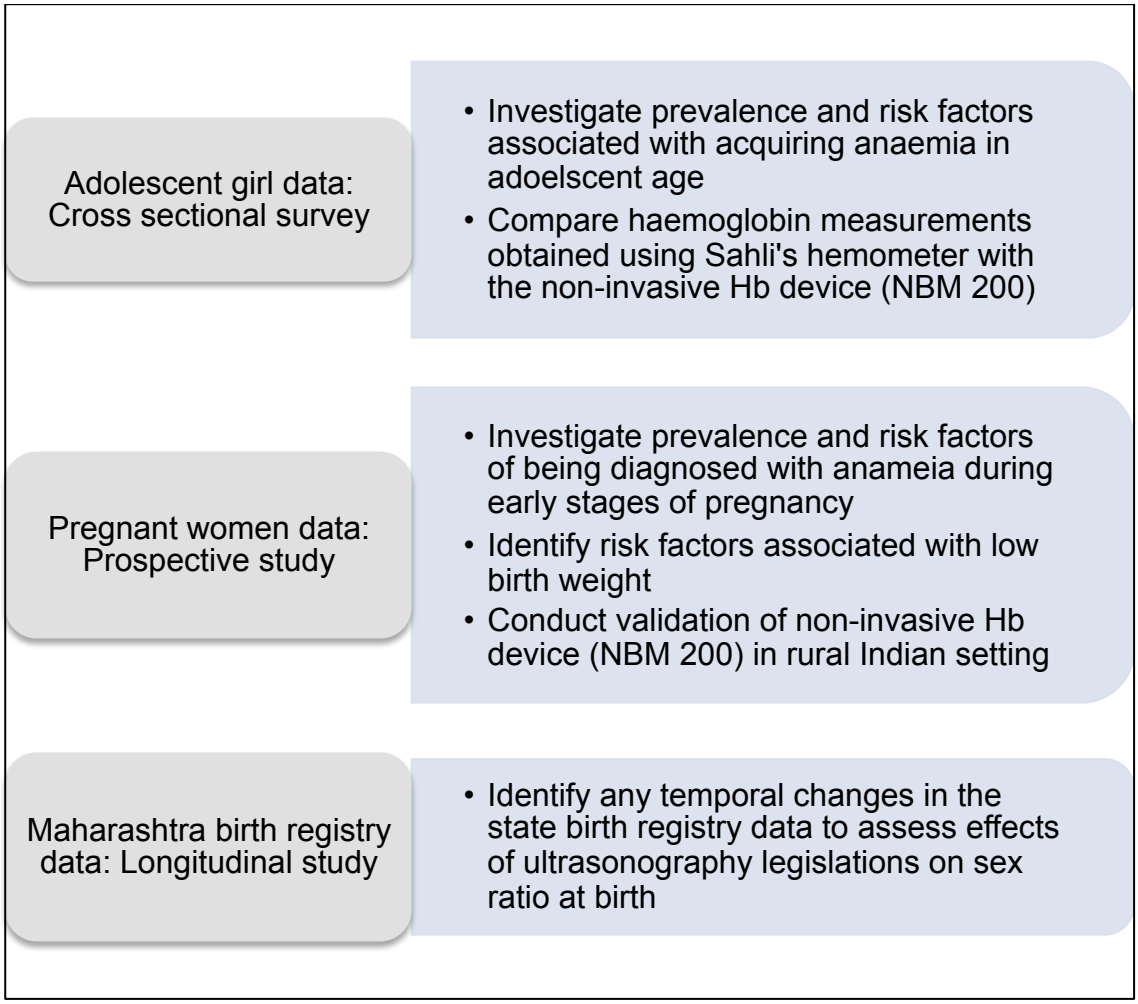
Secondary objectives

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.

(Please see the following page for figure 2)

Figure 2: Maharashtra anaemia study components with objectives



Recommendation 2

Limitations: Expand the limitations section in each results chapter to cover more thoroughly relevant effects of chance and power, bias, residual confounding, reverse causality etc.

Response: *To address this suggestion, I have revised limitation section for all 3 studies. The revised write-up of each study is outlined below.*

Changes (page numbers: 102 to 104)

Study limitations for Adolescent girls

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.

Changes (page numbers: 169 to 170)

Study limitations for pregnant women

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.

Changes (page numbers: 206 to 207)
Study limitations for sex ratio analysis

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.

Recommendation 3

Explanation: There are few places where assumptions were made why variables were being considered (eg which risk factors), and the methodology of the Joinpoint technique, and these require explanation, possibly using appendices.

Response: *I have now included a separate appendix (appendix 9) explaining selection of each individual level variable for the regression purposes. The same is referred in the main text. An additional appendix is included on the JoinPoint methodology to provide information and further references for readers (appendix 11). All appendices in the text and table of content are updated. I have outlined both appendices below for your kind reference*.

Changes (page numbers: 295 to 300)

Appendix 9: Individual level 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	Categorical	Yes	No	Published research (including a study from MAS) showed that BPL

	families (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	Categorical	Yes	No	Only one variable to

	in past 12 months				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.

Changes (page numbers: 305)

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

Recommendation 4

Confidence intervals: This should be added to show the precision of the estimates (both AG and PW chapter).

Response: *I have calculated confidence intervals for both chapters (adolescent girl and pregnant women). This is included in the descriptive results of respective chapter. The same is outlined below for your reference.*

Changes (page numbers: 76)

a) For adolescent girl study (chapter 3)

(N=1010)
Adolescent girls (anaemia: categorical)
Prevalence of anaemia in adolescent girls: 87.0%
95% Confidence intervals: 85.0% to 89.2%

(N=1010)
Adolescent girls (Hb: continuous)
Mean Hb: 10.1 g/dl
95% Confidence intervals: 10.0 g/dl to 10.2 g/dl

Changes (page numbers: 133 and 153)

b) For pregnant women study (chapter 4)

(N=287)
Pregnant women (anaemia: categorical)
Prevalence of anaemia in pregnant women: 77.0%
95% Confidence intervals: 72.1% to 81.9%

(N=287)
Pregnant women (Hb: continuous)
Mean Hb: 10.0 g/dl (N=287)
95% Confidence intervals: 9.9 g/dl to 10.2 g/dl

(N=248)
Low birth weight (LBW: categorical)
Prevalence of low birth weight: 7.0%
95% confidence intervals: 4.0% to 10.5%

(N=248)
Birth weight (BW: continuous)
Mean: 2.89 kg
95% confidence intervals: 2.8 kg to 2.9 kg

Recommendation 5

Recommendations: More specific recommendations about the way forward in addressing high prevalence of anaemia/additional areas.

Response: *I have included additional recommendations, and also reorganised the write-up to improve clarity. The revised recommendations are outlined below.*

Changes (page numbers: 212 to 219)

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

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.

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.

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.