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Research in collaboration with Halo Medical Foundation, India and University of Nottingham, UK

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[RESEARCH PROTOCOL]

This document provides detail introduction, aims, objectives and methodology of research project proposed for a PhD with the Division of Epidemiology and Public Health of the University of Nottingham, United Kingdom.

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A) Research title

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

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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 community risk factors for being diagnosed with anaemia in populations of pregnant women and adolescent girls living in forty villages in the Marathwada Region of Maharashtra. Additionally, the study will use the birth registry record of Osmanabad and Sholapur districts in Maharashtra to analyse temporal changes in 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 outline the background, rationale, aims, objectives and methodology of the proposed study. The methodology section will focus on project area, outcome measures, data collection and analysis methods. Ethical considerations, research limitations and dissemination plans will be 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 97 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 50% of pregnant women delivered under medical supervision and only 68% received antenatal care (3) (4). Nearly 75% of pregnant women were found to be anaemic with nearly 20% 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 also 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 an international NGO working in two blocks of Osmanabad district namely Tuljapur and Lohara since 1993 covering a population of about 100,000 individua;s. Much of the work of HMF is in maternal health, village healthcare networks, micro-financing, violence prevention, education and women's empowerment. The foundation also works in two blocks of Sholapur districts focusing on a gender equity programme and an urban 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 40-60 healthcare staff within 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 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 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 community 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 last three decades 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 Sholapur and Osmanabad districts to study temporal changes in sex ratio over a period of at least 32 years (1980-2012) and a further comparison could be made between rural and urban settings. This study focuses on a rural Indian population to address this evidence gap.

• 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 community level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 40 villages of Osmanabad district, Maharashtra using an observational epidemiological survey.

2. To study temporal changes in the sex ratio over a period of thirty-two years (1st January 1980-31st December 2012) in Osmanabad and Sholapur districts using anonymised routinely collected health care data.

H) Methods

1) Study design

1) For the first objective, a retrospective (January 2008- December 2012) and prospective cohort design (March 2014- June 2015) will be used for adolescents and pregnant women respectively.
2) For the second objective (sex ratio analysis), a time-series analysis will be conducted.

2) Study settings and population

The study will be conducted in 40 villages within the project area of HMF and cover a population of about 50,000. We expect approximately 3000 adolescent females and 800-900 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 1st March 2014 to 30th June 2015

Adolescent females:
- Aged between 13-17 years old
- Resident of the village specified in the research area

Sex ratio:
- Records between 1st January 1980 to 31st December 2012
- Location of birth: Osmanabad or Sholapur district based hospitals
- Private or Government hospital

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

5) **Outcome data**

Anaemia and Diabetes measurements: For adolescents, blood haemoglobin levels will be measured using Sahli's haemometer and also a non-invasive haemoglobin monitor that uses a simple finger probe. For pregnant women, blood haemoglobin levels will be measured using an automated haematology analyser (Sysmex company XP-100 model) and also using a non-invasive haemoglobin monitor that uses a simple finger probe between 12 to 20 weeks pregnancy. Additionally, blood glucose level will be assessed through biochemistry analyser only for pregnant women. Most women will only have one measurement but multiple measurements will be recorded where possible if relevant to their routine care. The week of pregnancy for any measurements made will be recorded along with the following parameters for each woman:

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)

Blood glucose level will be made available through biochemistry analyser (applicable only to pregnant women).

Pregnancy and neonatal health outcomes

1) Delivery outcome (spontaneous abortion/induced abortion/stillbirth/live birth/neonatal and infant death)
2) Birth weight (continuous 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)

Sex ratio data (for data from local hospitals 1st January 1980- 31st December 2012)

- Number of male live births in each year
- Number of female live births in each year
- Maternal age
- Annual rainfall of geographic region

6) Sample Size Calculation

As this is a pilot 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**.** We aim to collect data minimum from 1000 participants.

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. 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 community level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 40 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 community 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 4th standard, secondary education: until 7th standard, high school: until 10th, higher secondary education: until 12th 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 from 0-5/5-12/12-18/18 above, 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), Midarm circumference (cm), Body Mass Index, Blood pressure, Blood investigation (cell counter machine and non-invasive Hb unit for pregnant women/Sahli's haemometer and non-invasive unit for Adolescent girls), 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), Tobacco intake (Yes/No), If YES, tobacco form, frequency.

(Height, weight and mid-arm circumference will be recorded by the primary investigator AA during field visit for data collection, and equipment will be supplied by the HMF hospital. Weight will be taken using digital scales for maximum accuracy, height using a standard printed scale and mid-arm circumference by a measuring tape. 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. For pregnant women, Blood investigations will be conducted by a cell counter at the foundation's hospital and printed reports will be attached with the data collection form. While for adolescent girl, Sahli's haemometer will be used for field based testing and haemoglobin will be recorded on data collection forms. For both the groups, non-invasive machine will be used at field level and haemoglobin readings will be noted. 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).

Obstetric history (not applicable for adolescent girls): 1) Stillbirth 2) Spontaneous/induced abortions 3) Live children 4) Menstrual cycle related issue (regular/irregular) 5) Bleeding during menstrual cycle (normal/ moderate/ excessive/ unaware) 6) Hospital visits for obstetric issues (month/year) 6) 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 (4) Cup of tea (5) Cup of Coffee (6) Rice (7) Roti/Bread
Non-Vegetarian food (per typical week): (8) Eggs (9) Chicken (10) Meat (11) Fish

Assets/Socioeconomic status

Two wheeler, four wheeler, farming land, livestock, mobile phones, television sets, income and other assets

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? Can you name them?

Community risk factors for anaemia

Community 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 two: *To study temporal changes in the sex ratio over a period of thirty-two years (1st January 1980- 31st December 2012) in Osmanabad and Sholapur districts using anonymised routinely collected health care data.*

Information on sex from registered live births will be collected from at least from hospitals in the Sholapur and Osmanabad districts. If available, then Data will be collected from existing registration system in government records, which will have all births (hospital or home) in specified duration.

7) Data analysis

Statistical analysis will be conducted using Stata version 12. 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 community level risk factors for acquiring anaemia among adolescent females (13-17 years) and pregnant women residing in 40 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 (1st January 1980- 31st December 2012) in Osmanabad and Sholapur districts using anonymised routinely collected health care data.*

A time-series design using ecological (population level) data will be employed. The yearly number of male and female births for 32 years (1980-2012) 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. An interrupted time series analysis will be conducted to analyse temporal changes in sex ratio as a proxy to assess the impact of this legislation on female foeticide. 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 Maharashtra or India. Furthermore, community 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 trained staff involved in

the data collection and oral consent will be recorded on behalf of illiterate participants by project staff in the presence of a literate witness who acts 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 simple 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 previously outlined. 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 the local healthcare worker in each village. Data management such as data entry to computer systems, data cleaning and data analysis will be conducted at the Hospital administrative office. Data will be handled only by AA and later will be shared with project supervisors in the UK. The proposed research milestones are included in the appendix section, which includes permission letters from HMF, list of villages, research centre address, available infrastructure and research timelines.

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 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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