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Manuscript title

Access to a piped water supply is positively associated with haemoglobin levels in females living in rural Maharashtra State, India

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Short title

Living environment and adolescent anaemia in rural India

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Financial Disclosure: Donors/organisations/individuals listed above (other than MAS 1 research team) had no role in defining the study hypothesis, data collection, analysis, interpretation, or manuscript preparation.

Ethics statement: The study was approved by the Institutional Ethics Committee of the Government Medical College Aurangabad, Maharashtra, India (Reference number: Pharma/IEC/GMA/196/2014), and the Medical School Ethics Committee of the University of Nottingham, UK (Reference number: E10102013). All participants and their guardians provided signed informed consent for the survey and blood withdrawal separately. Other than those who declined to participate, all adolescent girls received a standardised health report including information on their haemoglobin level and anaemia status along with facilitated access to educational materials on anaemia through the health NGO, Halo Medical Foundation's (HMF) village-based services. Participant health reports were also provided to the village health worker/government nurse with arrangements for free consultation and assistance if any significant health problems requiring further assessment or treatment were identified during the study. HMF's hospital was also made available for free consultation as a primary referral centre if more specialist assessment or treatment was needed. On completion of data collection, an additional reminder letter was issued to village health workers indicating details of each severe anaemic case in their village to ensure that necessary medical advice and treatment was available.

Author Contributions: The Maharashtra Anaemia Study Phase 1 was designed by Dr Andrew Fogarty, Dr Anand Ahankari, Dr Puja Myles, and Dr Laila Tata. This specific study hypothesis was designed by Dr Anand Ahankari. Anand obtained the MAS Phase 1 data and conducted the analysis. All 3 authors (ASA, LJT, AWF) participated in the data analysis, manuscript preparation and approved it for the submission.

Conflicts of Interest: Authors have no conflicts of interest to disclose that are relevant to this study.

Abstract

Objectives: There is a high prevalence of anaemia in individuals living in rural India which may be modified by a number of environmental factors. The association between access to water, toileting facilities and healthcare services with prevalence of anaemia was explored to determine potentially modifiable community-level risk factors.

Study design: Cross sectional survey

Methods: Data were collected from adolescent females (13 to 17 years) living in 34 villages in rural areas of the Maharashtra State of India on measures of sanitation facilities and access to healthcare along with haemoglobin measurements. Linear and logistic regression analyses were conducted to investigate associations between environmental (community) factors and adolescent haemoglobin levels and anaemia respectively.

Results: Data were available from 1010 individuals which represented a response rate of over 97% of those who were approached for the study. The prevalence of anaemia was very high (87%) when measured using haemoglobin levels. Access to a piped water supply was associated with 0.59 g/dL of increase in haemoglobin levels (95% Confidence Interval (CI): 0.10 to 1.09). Associations between access to communal toilets, travel time to the hospital, health centres or nurses and haemoglobin levels or anaemia were not statistically significant.

Conclusions: Anaemia prevalence was very high in our study population. Simple improvements such as provision of regular piped water is associated with an increase in haemoglobin levels in rural Indian females. These are consistent with the hypothesis that chronic exposure to higher levels of microbes in the living environment contributes to the risk of anaemia.

Keywords: Haemoglobin, Anaemia, Adolescent, India, Risk factors

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Table: 1

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References: 10

1 **Introduction**

2

3 Communities living in rural India face a series of challenges to their well-being, that inhibit
4 their ability to thrive and fulfil their potential. These include a high prevalence of anaemia,
5 which has persisted despite the introduction of a national programme of iron and folic acid
6 (IFA) supplementation for adolescent females (1).

7

8 It is possible that some of the adverse features in the rural living environment in India may
9 be contributing to the communities' risk of having anaemia. These include limited access to
10 sanitation facilities, unclean or intermittent water for drinking, and sporadic access to
11 healthcare. These factors can be expected to increase the risk of exposure to microbes, as
12 well as impeding access to timely diagnosis and treatment of infection. Chronic infection and
13 inflammation are risk factors for anaemia that are poorly understood (2), especially in the
14 context of low- and middle-income countries. As access to sanitary toilets, reliable clean
15 water and healthcare are all amenable to intervention and in line with the sustainable
16 development goals (SDGs) as defined by the United Nations (3), these represent potentially
17 reversible risk factors for anaemia, that have the potential to improve the health of
18 communities for a relatively small investment.

19

20 Using data from a survey of anaemia in 34 villages in rural Maharashtra, India, the
21 association between access to water, toileting facilities and healthcare services with
22 prevalence of anaemia was explored to determine potentially modifiable community-level
23 risk factors.

24

25

26 **Methods**

27

28 *Study population*

29 A cross sectional survey was conducted between April 2014 and June 2015 in 34 villages
30 from two blocks of Osmanabad district of the Maharashtra state of India, having a total
31 population of approximately 65,000 individuals. Unmarried 13 to 17 years old adolescent
32 females from the study area were eligible to participate.

33

34 *Data collection*

35 The data collection has been described in detail elsewhere (4,5). In brief, data were collected
36 on individual lifestyle, anthropometric measurements, and blood haemoglobin levels were
37 measured using Sahli's Hemometer. Data were also collected on environmental factors from
38 all 34 villages with a particular focus on local healthcare and sanitation facilities using a
39 validated tool by trained research assistants. All data were recorded manually during field
40 research visits and then entered on computer system. All electronic entries were verified by
41 two members of the research team. Ethical approvals to conduct this research were
42 obtained and details are included in the ethics statement.

43

44 *Statistical analysis*

45 Anaemia was defined as a haemoglobin measurement less than 12.0 g/dL. The analysis
46 tested the hypothesis that the following community-level environmental exposures were risk
47 factors for anaemia; piped water supply in the village, daily water provision to individual
48 houses, access to communal toilets, access to a government health centre, access to
49 haemoglobin testing, and time to travel to a government primary healthcare centre.

50 Two models were built: analysing the association of environmental exposures with anaemia
51 using logistic regression and with blood haemoglobin levels using linear regression. The
52 initial analysis of the survey data (4) had identified that age, mid-upper arm circumference,
53 iron folic acid intake and fruits/fruit juices consumption were independent risk factors for
54 anaemia in this population, therefore these were considered a priori potential confounding
55 factors and adjusted for in all regression models, along with the use of robust standard errors
56 to adjust for clustering of exposures at the village level. As socio-economic status (SES) in
57 India is a potential confounding factor in analysis of the association between the
58 environment and risk of anaemia, two additional sensitivity analyses using two separate
59 measures of SES were applied to any community-level environmental exposures that were
60 statistically significant in the final model. The SES measures were self-reported; parental
61 agricultural land ownership and Below Poverty Line status, which is determined by the Indian
62 government for eligibility to support from the State. Data analysis was performed using Stata
63 16 (StataCorp, College Station, Texas, USA).

64

65

66 **Results**

67

68 1035 adolescent females from 34 villages were approached, of which 1010 consented and
69 provided full data (response rate > 97%, Supplementary 1). There was a very high

70 prevalence of anaemia (87% had anaemia, haemoglobin measurement < 12.0 g/dL). The
71 mean haemoglobin at the village level was 10.11 g/dL (Standard deviation 1.34 g/dL, Range
72 5 to 14 g/dL, ANOVA test, $p < 0.001$), and is presented in Supplementary 2.

73

74 The main analysis is presented in Table 1. Access to a piped water supply was associated
75 with 0.59 g/dL of increase in haemoglobin levels (95% CI: 0.10 to 1.09, $p = 0.01$). This
76 persisted after adjustment for socio-economic status as measured by parental agricultural
77 land ownership (+0.59 g/dL, 95% CI: 0.10 to 1.08, $p = 0.01$) or Below Poverty Line status
78 (+0.59 g/dL, 95% CI: 0.10 to 1.08, $p = 0.01$).

79

80 There were no statistically significant associations between access to communal toilets,
81 travel time to the hospital, health centres or nurse haemoglobin testing and haemoglobin
82 levels or anaemia.

83

84 **Discussion**

85

86 This is one of the first analyses to explore the association between the living environment
87 and risks of adolescent anaemia in a rural population living in Maharashtra, India. Availability
88 of piped water supply was associated with an increase in haemoglobin levels, and this
89 association remained after adjusting for markers of socioeconomic status.

90 The study response rate of over 97% was high, thus minimising bias in the data collected.

91 However, our study has some limitations. Haemoglobin values were estimated using a
92 Sahli's Hemometer, which is a manual technique that is appropriate for the research
93 setting but has more measurement error than automated blood analyser. However, any
94 bias that may result from this would be systemic and apply to the study population and
95 would not modify our results. As this is the secondary analysis of the existing dataset, our
96 analysis was relatively underpowered, with only 34 villages that provided the unit of
97 exposure. Nonetheless, having observed a significant association despite this limitation
98 suggests that the associations observed may be real and of a relatively large size of effect.

99 Adjusting for socio-economic status (SES) in rural India is challenging (5,6), but the use of
100 distinct two measures of SES gives us confidence that the positive association between
101 the provision of piped water and haemoglobin levels is not confounded by affluence. As
102 this was the secondary analysis of an existing dataset, no formal power calculations were
103 available. There are no data on infections and worm infestations, and that these may be

104 either confounding factors or on the causal pathway of the association between sanitation
105 and anaemia.

106

107

108 Our study area involves rural communities where village infrastructure is limited, thus not all
109 communities have water supply systems. Piped water supply mainly includes water supply
110 through an underground network of pipes installed and maintained by local government
111 authorities, through which water is supplied from a communal resource directly into
112 individual dwellings. In absence of such infrastructure, water for drinking and also for general
113 use is collected from communal source, primarily by women and young females. Access to
114 reliable clean water supplies can improve sanitation practices and is associated with a lower
115 risk of anaemia. It is biologically plausible as both will result in lower levels of chronic
116 infection, which is associated with anaemia (2).

117

118 It is apparent that basic sanitation facilities are essential for personal hygiene to reduce the
119 chances of gastrointestinal infections and also vital to improve general health and wellbeing.
120 Overall, our findings are consistent with research published from India and other developing
121 countries. A survey from rural areas of Kerala state of India of 257 adolescent females
122 reported that hand washing practice after toileting and before meals were protective factors
123 against anaemia (7), where access to water is vital to improve personal hygiene. Analysis
124 from the National Family Health Survey (NFHS 3, 2005-06) of India reported that poor
125 household facilities and conditions (such as lack of toilets) were associated with increased
126 risk of anaemia in young children (8). Further, analysis of the NFHS 4 (2015-16) data from
127 15 to 49 years old Indian females showed that lack of improvements in water source (Odds
128 Ratio [OR] 1.12) as well as toileting facilities (OR 1.14) were associated with an increased
129 risk of anaemia (9). Similar results were seen in other developing countries include Nepal
130 (OR 1.59) and Timor-Leste (OR 1.11) where lack of improvements in water facilities
131 (includes lack of piped water supply into individual houses) were associated with an
132 increased risk of anaemia (9). Access to water promotes frequent hand washing and also
133 improves hygiene by reducing chances of infections (such as soil-transmitted helminthiasis),
134 which directly lowers anaemia risks (10).

135

136 In summary, the prevalence of anaemia in a population of adolescent females living in rural
137 India was high, and adolescent females living in communities with piped water supplies had
138 higher haemoglobin levels. This is unquestionable an important common good that are

139 amenable to interventions, so ideally studies before and after the provision of such facilities
140 could determine if the association is causal, ideally using measures of systemic inflammation
141 and the microbiological burden of exposure in the living environment to help investigate the
142 causal pathways involved. Further investigations into environmental factors along with
143 socioeconomic status, IFA supplementation, household resources, parental education and
144 anaemia investigations on adolescent females will help identify causes of high prevalence
145 of anaemia in India to inform future research, intervention strategies and policies.

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Table 1: Association of community-level risk factors with haemoglobin level and anaemia

Village Characteristics	Number of villages (%)	Haemoglobin, g/dL (95% CI)	p value	Risk of anaemia OR (95% CI)	p value
Piped water supply					
No	10 (30)	0	0.01*	1	0.11
Yes	24 (70)	+0.59 (+0.10 to +1.09)		0.28 (0.05 to 1.37)	
Daily household water provision (at least once a day)					
No	7 (21)	0	0.76	1	0.25
Yes	27 (79)	-0.08 (-0.66 to +0.49)		0.49 (0.14 to 1.66)	
Access to communal toilets					
No	23 (68)	0	0.81	1	0.43
Yes	11 (32)	+0.06 (-0.51 to +0.64)		0.60 (0.17 to 2.15)	
Access to government health centre					
No	24 (70)	0	0.81	1	0.54
Yes	10 (30)	-0.06 (-0.67 to +0.54)		1.51 (0.39 to 5.83)	
Government nurse conducts haemoglobin testing					
No	8 (24)	0	0.44	1	0.59
Yes	26 (76)	-0.23 (-0.86 to +0.38)		1.49 (0.33 to 6.63)	
Travel time to the nearest government's Primary Health Centre (minutes)	Continuous variable	-0.00 (-0.02 to +0.01)	0.74	1.00 (0.96 to 1.04)	0.74

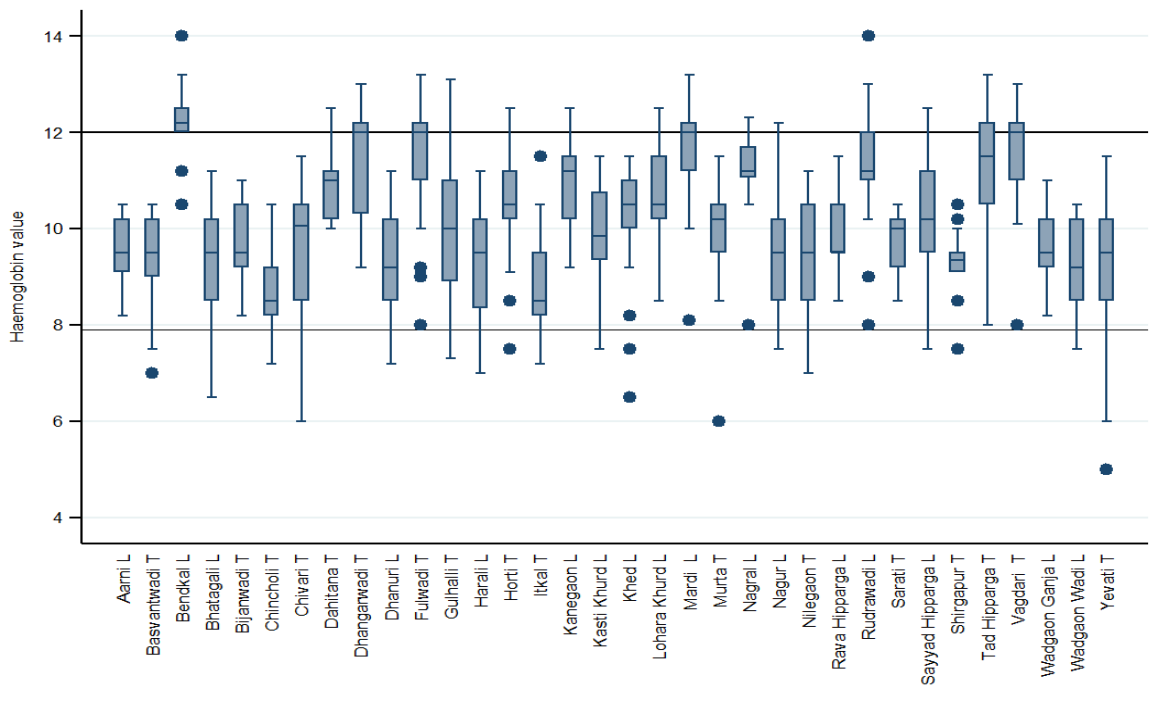
Table 1 footnotes:

- Anaemia was defined as a haemoglobin level below 12.0 g/dL.
- For linear regression, haemoglobin (Hb) values were used as a continuous measure (Hb measurement-primary outcome of interest) and for the logistic regression binary categorical outcome was used (anaemic or non-anaemic status).
- Analysis presented in the table is adjusted for priori potential confounding factors associated with anaemia (4) along with the use of robust standard errors to adjust for clustering of exposures at the village level.
- A priori potential confounding factors were as follows; age (continuous variable in years), mid-upper arm circumference of dominant hand (binary categorical variable in cm), currently consuming Iron Folic Acid supplements (binary categorical variable) and fruits/fruit juices intake (binary categorical variable).
- Percentages rounded to the nearest whole number. Statistical significance determined as *p<0.05.

Supplementary 1: Research area environment and resources

- The Maharashtra Anaemia Study Phase 1 (MAS 1) included 34 villages having approximately 65,000 population. All villages had access to government nurses, however most of them were visiting only once a month (N= 29).
- Nurses in our study area were primarily ANMs (Auxiliary Nurse Midwives) who were responsible to deliver antenatal services, conduct haemoglobin investigations, iron folic acid tablet distribution along with other healthcare and social duties. However, their primary focus was on pregnant women with limited time and services available to non-pregnant women and adolescent females.
- Of 34, nine villages had government health centres (where a nurse was stationed full-time, known as sub-centre in India), as a result, adolescents in these villages had better access to a nurse than others.
- Only one village had a Primary Health Centre (PHC) with 24 hours emergency service facility with access to a medical doctor.
- Majority of the villages (N=33) had an ASHA (Accredited Social Health Activist) personnel assigned through the government's national health scheme.
- Other healthcare related workforce in villages included nutrition workers who were involved in running a nursery and providing nutrition supplies to pre-school children (<6 years) and lactating mothers.
- No villages had a centralised automated or manual water purification facilities, and the quality of drinking water provided was not objectively tested for contamination.
- Villages had limited transport services (mostly private operators). Government transport facility such as bus service was almost non-existent due to low levels of demand and higher use of private transport operators due to convenience.
- Ten villages had secondary schools (up to 10th standard, until 15-16 years of age), while the rest had primary schools (up to 4th or 7th standard depending on school size and government approval/funding).
- Two variables were used to investigate water supply at the village level as follows; (a) Piped water supply, and (b) Daily household water provision. Villages where piped water infrastructure is available, then the same is accessible to all residents once individual households register with local authorities. In absence of such underground infrastructure, a communal water source is used to fetch water for drinking and general usage. It is important to note that in some villages due to poor water management, or a lack of water reservoir even after such piped system, water may not be supplied, thus the second variable was included in the model (Daily household water provision). The outlined two variables measured village level resources rather than individual dwelling situation (such as household water connection). Only one village level data collection form was used to collect data from each village (34 village/units, thus 34 data forms in total).

Supplementary 2: Haemoglobin distribution across 34 villages



Supplementary 2 footnotes:

- $P < 0.001$ (ANOVA test)
- Villages are plotted on the X-axis and haemoglobin value (g/dL) is on the Y-axis. Village name is followed by block initials such as 'T' for Tuljapur block, 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 indicate outliers.