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NOTE

Diagnosing anaemia via smartphone colorimetry of the eye in a population of pregnant women

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Abstract

Objective. Screening for disease using a smartphone camera is an emerging tool for conditions such as jaundice and anaemia, which are associated with a colour change (yellowing in jaundice; pallor in anaemia) of the external tissues. Based on this, we aimed to test a technique to non-invasively screen for anaemia in a population highly affected by anaemia: pregnant women in India. In this group, anaemia can have severe health consequences for both the mother and child. *Approach.* Over 3 years of data collection, in 486 pregnant women in India, we attempted to replicate a previously successful smartphone imaging technique to screen for anaemia. Using smartphone images of the eye and eyelid, we compared two techniques (white balancing and ambient subtraction) to control for variation in ambient lighting, and then extracted 'redness' features from images, which we used as features to predict anaemia via statistical modelling. *Main results.* We found that we were not able to predict anaemia with enough accuracy to be clinically useful, at 89.6% sensitivity and 26.1% specificity. We consider the hypothesis that this may be due to pigmentation on the sclera and palpebral conjunctiva. Visual judgement showed that pigmentation on the sclera, which may affect the measured colour, is more prevalent in pregnant women in India than in preschool aged children in Ghana (a population previously studied in this context). When participants with subjectively judged visible scleral pigmentation are removed, ability to screen for anaemia using the smartphone images slightly improves (93.1% sensitivity, 28.6% specificity). *Significance.* These findings provide evidence to reinforce that applying smartphone imaging techniques to understudied populations in the real world requires caution—a promising result in one group may not necessarily transfer to another demographic.

1. Introduction

The smartphone has emerged as a tool for patient education (Sari *et al* 2022), symptom monitoring (Masterson Creber *et al* 2016), disease biomarker monitoring (Korot *et al* 2022), in-clinic communication (Wu *et al* 2010), and even monitoring the effect of process improvements on clinical workflows (Goel *et al* 2021). Smartphones contain high resolution cameras which can be characterized and used as precision imaging devices. This is appealing in healthcare, and has been a target of much research (Mannino *et al* 2018, Outlaw *et al* 2020, Suner *et al* 2021, Dimauro *et al* 2023, Kazankov *et al* 2023). In regular clinical practice, however, smartphone imaging for non-invasive diagnosis remains rarely used (Nixon *et al* 2019, 2020). One potential cause for the lack of adoption of smartphone imaging in the real world is the challenge of obtaining robust evidence across diverse healthcare environments and populations.

1.1. Disease targets for screening

Success using visible light images of the ocular surface—and the conjunctiva of the eyelid—for screening has been shown in jaundice, where an increased concentration of bilirubin in the blood leads to a yellowed appearance (Outlaw *et al* 2020, Nixon-Hill *et al* 2023). Promising results have also been shown in screening for anaemia, where a reduced concentration of haemoglobin within the blood leads to a reduced redness of the soft tissues (e.g. Dimauro *et al* 2023, Nixon-Hill *et al* 2023, Kasiviswanathan *et al* 2020). Anaemia is a particularly pertinent issue in antenatal care because it is prevalent, with around 35% of pregnant women in India having moderate or severe anaemia (Nair *et al* 2016). During pregnancy, anaemia can lead to reductions in birth weight, as well as increase the risk of pre-term birth (Haider *et al* 2013). Anaemia during pregnancy also increases the risk of maternal mortality (Brabin *et al* 2001). In fact, one study in 2009 estimated that anaemia during pregnancy may be responsible for 40% of maternal deaths (Kalaivani 2009). It is therefore apparent that it would improve patient outcomes to have an accessible screening tool to identify anaemia during pregnancy (Hämäläinen *et al* 2003).

In this research, we aimed to non-invasively screen for anaemia in pregnant women in India, using a previously published technique which had been tested in a population of preschool-aged children in Ghana (Wemyss *et al* 2023).

Anaemia screening using images of the eye depends upon having a way to accurately assess the redness of the eye. This is challenging outside the laboratory because the colour of the pixel in the obtained image depends upon the spectral power distribution of the light reflected from the sclera and blood, which in turn depends on both the illumination, and the surfaces themselves. Illumination varies greatly between settings and times of day. Studies have attempted to avoid this effect by controlling the lighting, typically with a shield and a known light source (Kent *et al* 2000, Bevilacqua *et al* 2016, Jain *et al* 2020, Suner *et al* 2021, Dimauro *et al* 2023). Other studies have used a white reference within the image, which allows them to identify the colour of the illuminant, and correct for the lighting (Suner *et al* 2007, Lobbes *et al* 2019), or rely upon inbuilt image processing in the smartphone camera to adjust for varying lighting conditions (Chen *et al* 2016, Collings *et al* 2016, Mannino *et al* 2018). Further studies have demonstrated good results by training their algorithm on images with a variety of white balances, to attempt to produce algorithms which are believed to be more robust to ambient lighting and white balance (Kasiviswanathan *et al* 2020). There is, however, evidence to suggest that these techniques can be avoided using a technique called ambient subtraction (Nixon *et al* 2020, Outlaw *et al* 2020). Ambient subtraction relies upon taking two images; one image is taken with the camera flash (and ambient lighting), and one is taken without (only ambient lighting), and has been shown to perform well on smartphone images (Nixon-Hill *et al* 2020). The difference between the images is approximately equal to the image under the flash alone—in effect, ambient lighting is subtracted.

Further processing is required to extract a feature which can be correlated against the blood haemoglobin concentration. There are a range of neural network-based techniques for this, but there are also at least intuitive two-colour metrics for assessing redness. These are the r-chromaticity (a measure of the red channel of the image relative to the overall brightness), and the erythema index (a logarithmic ratio of the red and green channels). These techniques have the benefit of being explainable. Such techniques are also expandable using image filtering. One example is through only considering the chromaticity of blood vessels, rather than the sclera as a whole (Dimauro *et al* 2023). Filtering for blood vessels may help to remove ocular features which could affect the measured colour—although it is typically assumed that these are very rare.

1.2. Factors affecting feasibility of screening

The patient-related features of ocular physiology which might affect screening are typically incidental findings with no direct pathological implication. These features may be highly localized or diffuse. Localized visible features include Axenfeld loops (von Axenfeld 1902), which are intra-scleral loops of the ciliary nerve, commonly surrounded by a small area of pigmentation (Reese 1931). More generally distributed features may include pigmentation of the conjunctiva. There is some preliminary evidence to suggest that pigmentation over the sclera may be more common during pregnancy (Liesegang 1994), possibly due to an increase in melanocyte-stimulating hormone (Jadotte and Schwartz 2010), and any such pigmentation may alter the results of colorimetric analysis, especially if it is highly variable between individuals. Pregnancy has even been shown to be linked to a decrease in the number of capillaries in the anterior segment, which could further affect the colorimetric properties (Chawla *et al* 2013, Yenerel and Küçümen 2015). There is, therefore, a possibility that normal physiological changes during pregnancy, such as changes in pigmentation, vasculature, and choroidal thickness (Goktas *et al* 2014), may change the apparent colour of the sclera. This would mean that the visible colour is no longer directly linked to the concentration of the underlying biomarker (the blood haemoglobin).

There are also pathological factors which may affect the ability to use the ocular surface for screening—for example, there is evidence to suggest that dry eye (Schechter *et al* 2002) may occur during pregnancy, which may cause inflammation that alters the appearance of the eye. An overview of visible features is shown in figure 1.

The effect of these features on image-based screening is understudied. We investigated using eye images to predict anaemia in pregnant women in India. In the process, we aim to determine whether results previously obtained in preschool-aged children in Ghana (Wemyss *et al* 2023) can be replicated, and to investigate the frequency of visible features of the ocular surface which might affect these results.

2. Materials and methods

This project was ethically approved by the Institutional Human Ethics Committee of All India Institute of Medical Sciences (IEC-479/02.08.2019, OP-36/06.11.2020). The research was conducted in accordance with the principles of the Declaration of Helsinki and local legal requirements. All participants gave written, informed consent to take part in the study. Participants gave consent for this study to use their data for research, but not to reproduce any identifiable photographs in the work—therefore only stylised drawings of images are shared in the published article, and the supplementary information contains image statistics, rather than images themselves.

2.1. Patient and public involvement

Aspects of data collection were informally guided in part by patient and public involvement (in particular, decisions around imaging and COVID-19 concerns). More patient and public involvement was considered as a follow-on from this study, in case the results of this study suggested this smartphone imaging system could be clinically useful. However, the results did not support use of the tool as it currently stands, and consequently no patient-public involvement was carried out.

2.2. Data collection

Outpatients were invited to participate when attending routine appointments between 8 September 2020 and 31 January 2023 at the Department of Obstetrics and Gynaecology at the All-India Institute of Medical Sciences, New Delhi, India, and the Antenatal Outpatient Department at the Comprehensive Rural Health Services Project Hospital, Ballabhgarh, India. Convenience sampling was used to select potential participants.

Patients were excluded for whom participation would have impacted clinical care, or who were unable to give informed consent, or who had health conditions affecting the appearance of the eye. Such conditions include, for example, conjunctivitis or uveitis. All participants were provided with an explanation of the purpose of the study, and gave written, voluntary, informed consent before involvement.

Age, gestational age, blood haemoglobin concentration, an estimate of whether the research clinician considered them to exhibit visible pallor, and the start and end times of their photograph acquisition were recorded for each participant. The time data were used to confirm that images were associated with the correct clinical data. The blood haemoglobin concentration was obtained from a finger prick blood sample, analysed by the HemoCue Hb 301 portable haemoglobinometer.

Images of one sclera and one lower eyelid were captured on a Samsung Galaxy S8 smartphone. Pairs of images were taken in short succession—once with the flash (built into the phone) on, and once with the flash off. Images were stored in an uncompressed binary format ('RAW') to minimize automatic software post-processing. Images were taken across a range of rooms and settings across the two sites; no specific adjustment was made to the lighting for imaging. In practice, this meant that images were taken in a well-lit room, with a combination of artificial lighting (fluorescent, incandescent, or LED, depending upon the room) which was—when the room had windows—supplemented by the sunlight entering through the window.

When images were taken, the participants were sat or stood upright, with their head unsupported. The camera was held by hand and was positioned as close to the participant as possible (while still allowing the camera to focus, and ensuring the entire eye was visible in the frame). This meant that the camera was typically between approximately 30 and 60 cm from the objects.

2.3. Data labelling

For each participant, a set of images which clearly showed the desired regions of interest were selected. The lower eyelid and the sclera were manually segmented by an observer who was not able to view the case report forms. Each image of the sclera was subjectively assessed by an observer for image quality features. These features, detailed below, are summarized in figure 1.

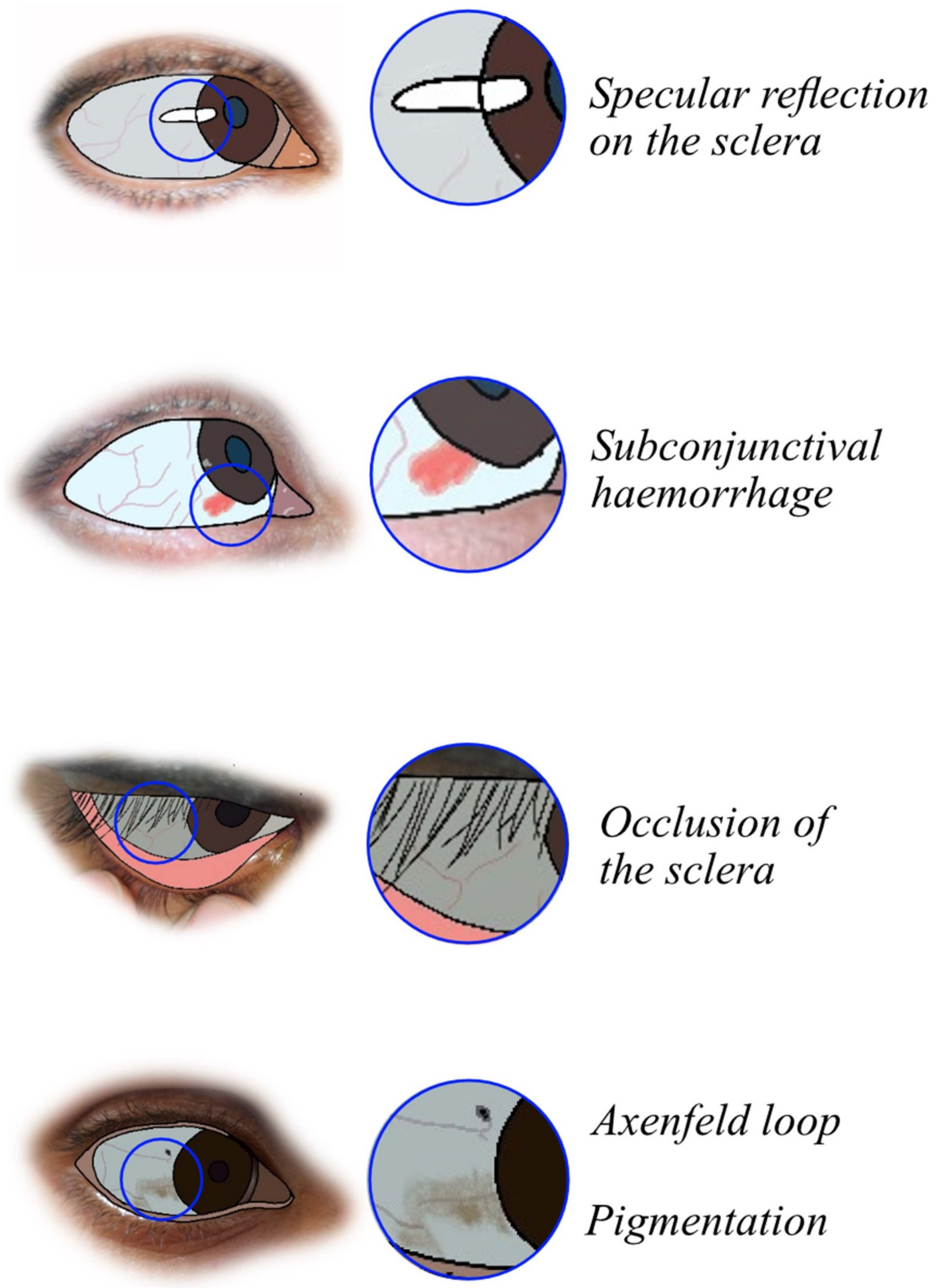

Figure 1. Examples (from the present dataset) of factors affecting image quality. The illustrations of the sclera are traced over the participant images and coloured using colours selected from the underlying areas of the image. A small border of the real participant image is shown for context. The full images could not be presented because consent was not gained for sharing any identifiable aspects of the images.

1. **Specular**—Was the specular reflection from the main illumination on the sclera (rather than the iris or pupil)? Specular reflection on the sclera itself may lead to a ‘bloom’ effect and oversaturation of surrounding areas of the sclera.
2. **Shadow**—Were there shadows or lines on the sclera?
3. **Reflections**—Were there clear reflections of the room or smartphone on the sclera?

4. **Pigmentation**—Were there patches of visible pigmentation, or dark patches due to Axenfeld loops, on the sclera?
5. **Occlusion**—Was the sclera occluded, for example by eyelashes or eyelids?
6. **Bleeds**—Was there redness on the sclera beyond expected distribution (e.g. due to subconjunctival haemorrhage).
7. **Focus**—Was over 50% of the sclera judged to be out of focus? The criterion for this was inability to clearly identify any blood vessels on the out of focus region of the sclera.
8. **Cysts**—Were there large, raised surface vessels or cysts on the sclera?
9. **Other**—Was there anything else obviously wrong with the image?

If conditions 5, 6, 7, 8, or 9 were met, the image was not included in the analysis.

2.4. Prediction of blood haemoglobin concentration

Sclera and lower eyelid images were investigated for their ability to predict blood haemoglobin concentration. For all patients with images which were deemed suitable for inclusion (see 'data labelling'), the methods from (Wemyss *et al* 2023) were replicated. As per (Wemyss *et al* 2023), two techniques were used for to correct for ambient lighting: either ambient subtraction (a technique validated in (Nixon-Hill *et al* 2020)), and sclera white balancing (which relies on specular reflection in the sclera providing insight into the illuminant). After this, 'redness' features (erythema index, r chromaticity, g-chromaticity, CIE 1976 a^*b^* chromaticity) were calculated for each region of interest (sclera, lower eyelid conjunctiva) in each image.

The best combination of features (one feature from the sclera, and one feature from the eyelid) was then selected—for regression on the basis of optimising correlation coefficient R, and for classification on the basis of optimising classification accuracy in a naïve Bayes classifier. The modifications to the published method in (Wemyss *et al* 2023) were that: (Sari *et al* 2022) no images of the lower lip were used (due to the COVID-19 pandemic), and (Masterson Creber *et al* 2016) no camera-specific calibration was used, because all images were captured on a single smartphone in this study.

3. Results

3.1. Dataset

A total of 486 pregnant women consented to participate, of whom 405 remained after image quality checks. The mean time taken to image one participant, as calculated from the digital timestamps attached to the images, was 68 s.

The dataset is summarized in figure 2. Median age was 25.0 years, with standard deviation of 4.40 years, and range 18.0–41.0 years. Median blood haemoglobin concentration was 10.3 g dl^{-1} (mildly anaemic), with a narrow distribution (standard deviation 1.71 g dl^{-1}), despite the large overall range from 4.3 to 15.2 g dl^{-1}. Pregnant participants were on average 23.8 weeks pregnant (median, with standard deviation 9.27 weeks). According to ordinary least-squares regression, there was a weak ($R = 0.182$) but statistically significant ($p < 0.0001$) negative correlation between gestational age and blood haemoglobin concentration. In the first trimester, mean blood haemoglobin concentration was 11.2 g dl^{-1} ($N = 62$), compared with 10.4 g dl^{-1} ($N = 167$) in the second, and 10.1 g dl^{-1} ($N = 250$) in the third.

3.2. Prediction of blood haemoglobin concentration

Methods from (Wemyss *et al* 2023) were replicated on the 405 participants with images of both regions of interest. As per the original study, whitebalancing performed best on the sclera, whereas the ambient subtraction technique performed best on the lower eyelid. After exclusion for a subtracted signal-to-noise ratio (Nixon *et al* 2019) less than 1.0, 378 participants remained. The best correlation for the sclera had $R = 0.275$ ($p < 0.000\,01$) against blood haemoglobin concentration, and the best correlation for the eyelid had $R = 0.309$ ($p < 0.000\,01$). These are approximately half the correlation coefficients obtained in preschool aged children in Ghana.

The best analysis (optimizing for classification accuracy at predicting blood haemoglobin concentration below 11.0 g dl^{-1}) combining the sclera and eyelid (where 378 participants remained after signal-to-noise ratio filtering) had a correlation coefficient of $R = 0.335$. This gave 95% limits of agreement against the measured blood haemoglobin concentration of $\pm$3.22 g dl^{-1}. These data are summarized in panels (a) and (b) of figure 3.

Sensitivity to detect anaemia (defined as <11.0 g dl^{-1} blood haemoglobin concentration) was 89.6% (95% CI from 84.3% to 92.7%), and the specificity was 26.1% (95% CI from 19.1% to 34.1%). The area under the curve was 0.648. Details on classification performance are presented in panel (a) of figure 4. All 12 severely anaemic participants (blood haemoglobin concentration below 7.0 g dl^{-1}) were correctly identified,

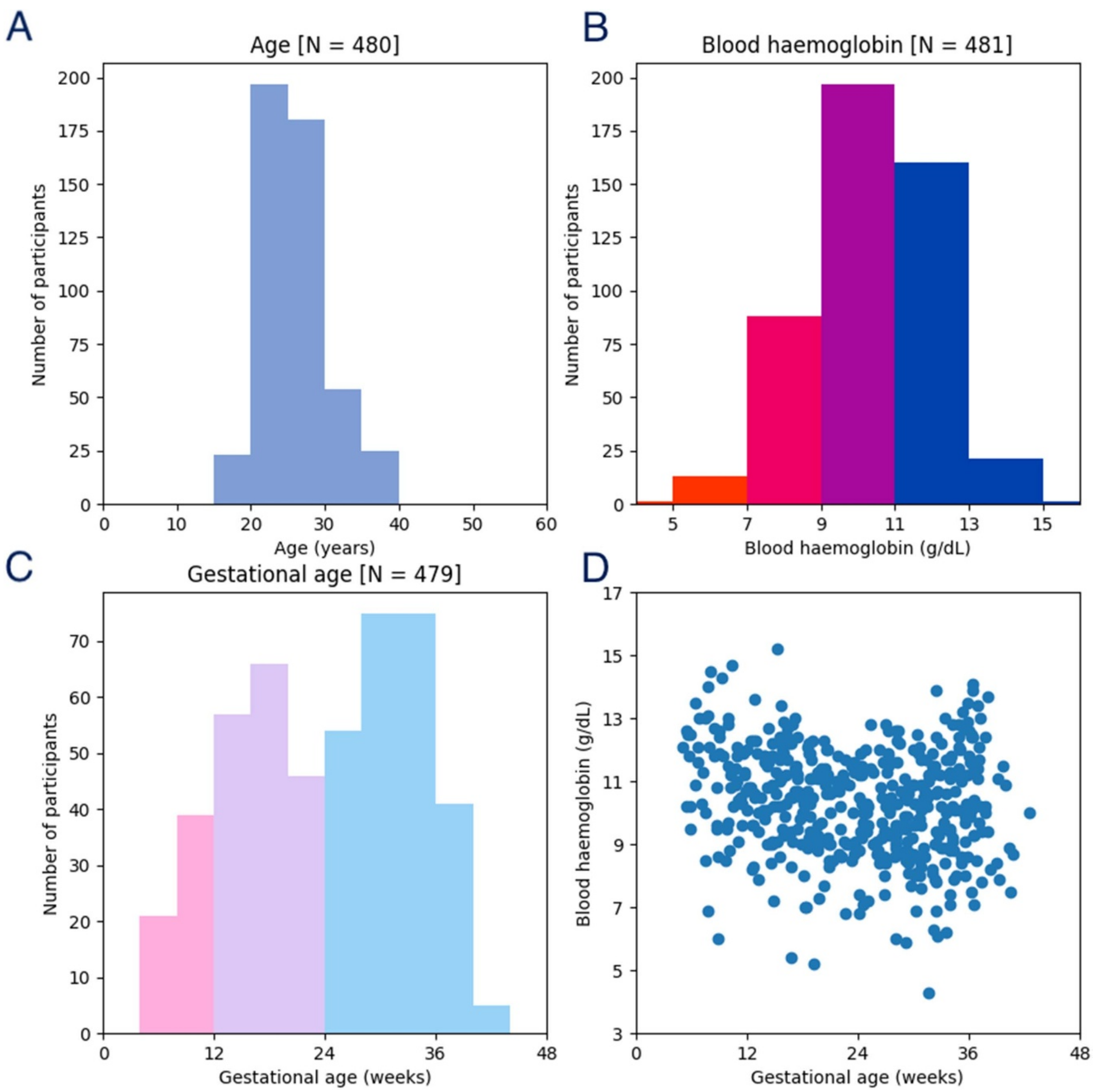

Figure 2. Summary of the dataset collected in this study. Note that the sample sizes for each individual feature are always less than the overall sample size due to missing data.

Table 1. Comparison of classification metrics quantifying ability to predict anaemia in the present population of pregnant women in India, compared to preschool-aged children in Ghana in (Wemyss *et al* 2023). AUC means area-under-the-curve.

Metric	India (current study)	Ghana (Wemyss *et al* 2023)
Mean age (years)	25.0 (stdev = 4.40)	1.25 (stdev = 1.65)
Regions	Sclera, Lower Eyelid	Sclera, Lower Eyelid, Lip
Sample size (N)	405	43
Sensitivity	89.6	92.9
Specificity	26.1	89.7
AUC	0.648	0.909

and 127 of 137 moderately anaemic participants (blood haemoglobin concentration below 10.0 g dl^{-1}) were correctly identified. However, 105 of the 142 non-anaemic participants were incorrectly predicted as being anaemic. The receiver operating characteristic curve is shown in panel (b) of figure 4. A comparison against (Wemyss *et al* 2023) is presented in table 1.

3.3. Sclera pigmentation

When pigmentation was subjectively assessed by eye, 238 patients (49% of the sample size) were judged as having visible pigmentation or Axenfeld loops on the scleral conjunctiva. This compared to just 9.67% in the population of preschool aged children studied in (Wemyss *et al* 2023). This supported the hypothesis that the change in demographic had changed colorimetric features of the sclera. There was no significant relationship

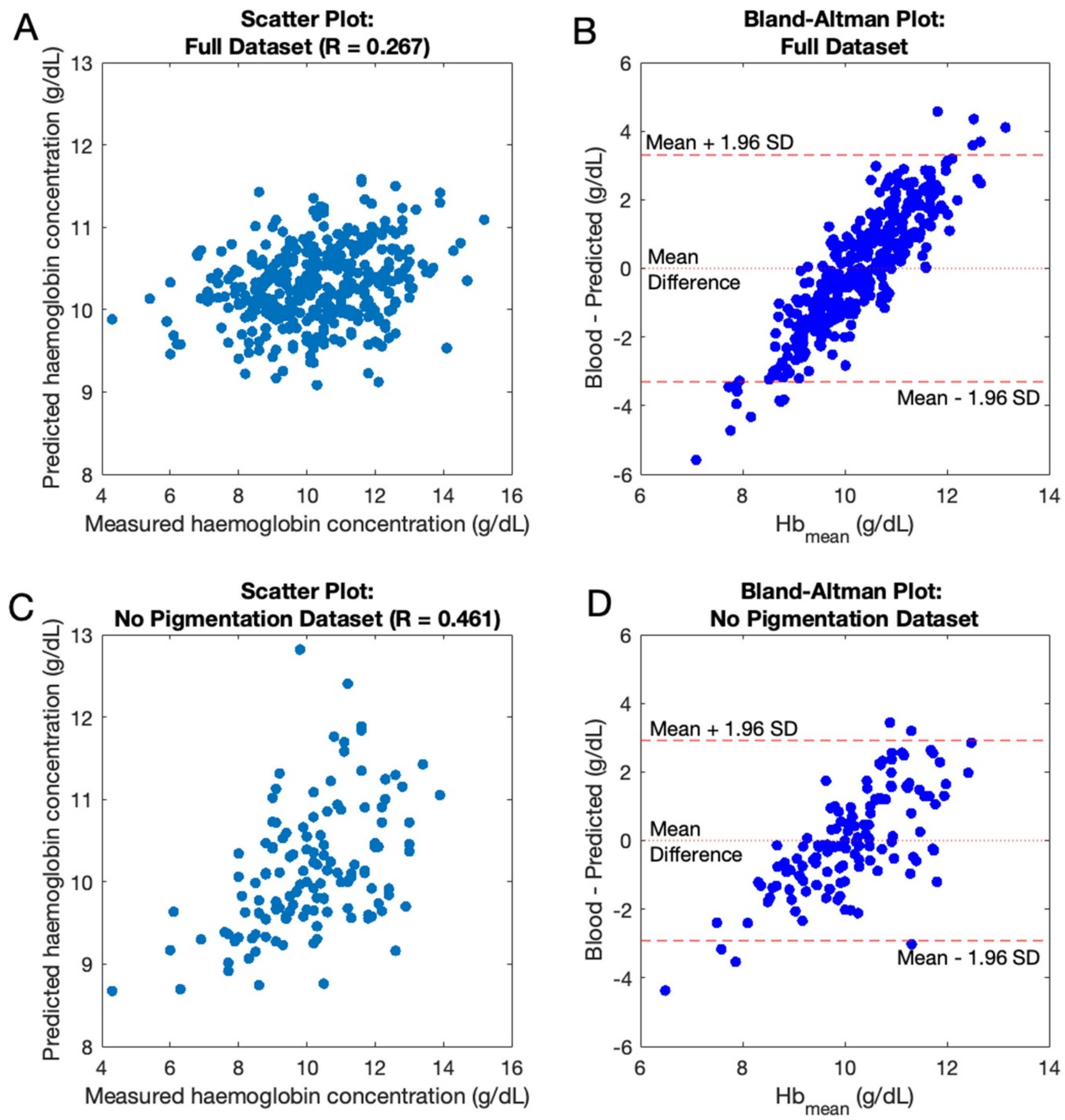

Figure 3. Left: scatter plot of measured blood haemoglobin concentration against the predicted blood haemoglobin concentration from the analysis pipelines of the sclera and lower eyelid with the best combined accuracy at screening for anaemia. Right: Bland–Altman analysis showing 95% limits of agreement between the predicted and measured blood haemoglobin concentrations. Top: the full dataset ($N = 378$). Bottom: the dataset when all participants judged by eye to have pigmentation on the sclera were removed ($N = 129$).

between the presence or absence of pigmentation, and the blood haemoglobin concentration (logistic regression, $p = 0.168$), or the gestational age ($p = 0.545$).

If all participants judged to have any visible pigmentation or Axenfeld loops on the sclera are excluded from the analysis, 129 patients remain who are suitable for analysis. When their blood haemoglobin concentration is predicted, the correlation coefficient is greater than for the entire dataset ($R = 0.461$) and this is reflected by a decrease in the 95% limits of agreement to 2.91 g dl^{-1} (figure 3, panels (c) and (d)).

This is not reflected by a large change in classification metrics. The area under curve was 0.639, sensitivity was 93.1% (95% confidence intervals from 85.6% to 97.4%), and specificity was 28.6% (95% confidence intervals from 15.7% to 44.6%). This may be, in part, due to the small number (42) of non-anaemic patients in the dataset. These data are shown in figure 5 panel (a), where all 5 severely anaemic participants were correctly identified. The receiver operating characteristic curve (panel (b) of figure 5) exhibits a quantization effect due to the reduced dataset size after all participants with visible pigmentation on the sclera were excluded.

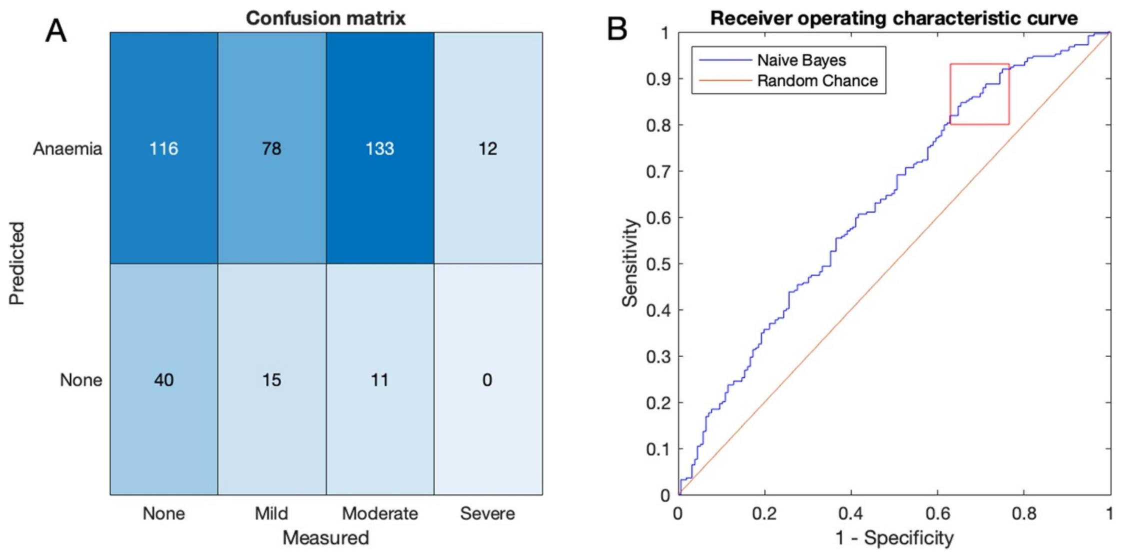

Figure 4. Performance at predicting anaemia on all participants with suitable images. The area under the curve was 0.648. The red square shows the area of the chosen threshold, at which sensitivity was 86.9% and specificity was 25.6%.

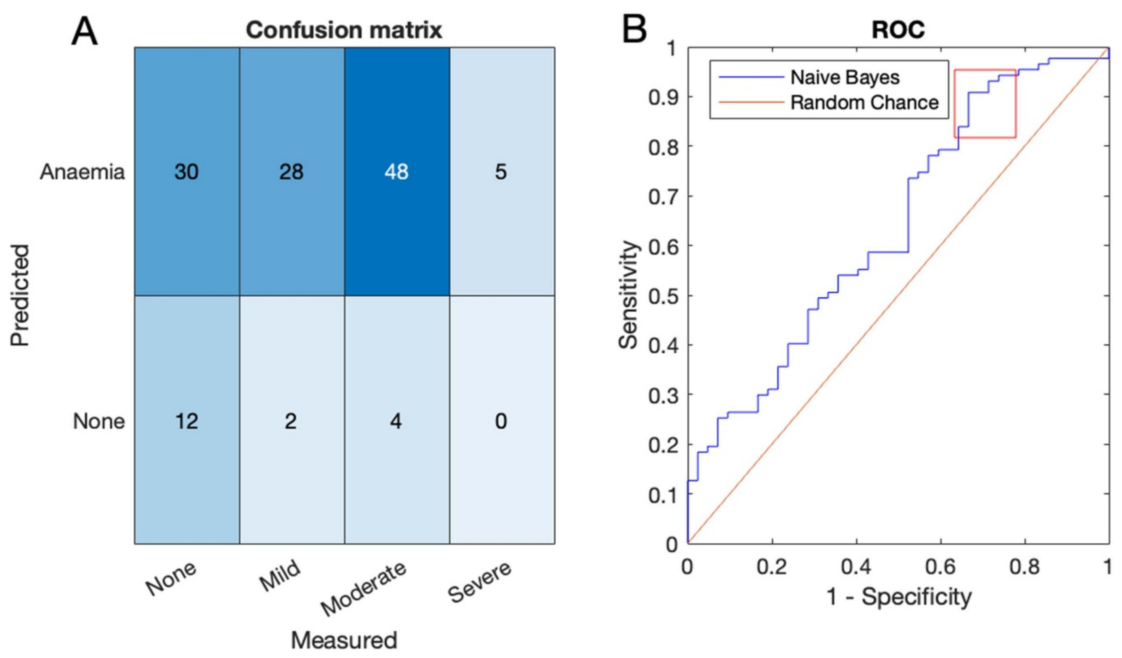

Figure 5. Performance at predicting anaemia on all participants with no visible pigmentation on the sclera, and suitable images. The area under the curve was 0.639.

4. Discussion & conclusions

Pregnant women in India have a high prevalence of anaemia (Kalaivani 2009). The participants in this study fit this characteristic, with the average participant having mild anaemia. We found the expected relationship between a longer duration of pregnancy and reduced blood haemoglobin concentration, although his was a weak effect, possibly reduced by iron supplementation increasing later in pregnancy.

4.1. Screening with a smartphone

As a potential clinical tool, smartphone imaging was found to be fast (taking just over 1 min per patient). However, we were unable to transfer into a population of pregnant women in India the successful screening results that we had obtained for detecting anaemia in preschool-aged children in Ghana. A comparison is presented in table 1.

In neither model that we tested were any severely anaemic patients misclassified. However, the specificity was low enough that this would not be an effective diagnosis tool, and it is possible that with a larger sample size, the sensitivity at detecting severe anaemia may change. Favouring false positives over false negatives is likely valuable, especially for severe anaemia and during the last trimester of pregnancy, where anaemia can have severe maternal and foetal outcomes (Akhtar and Hassan 2012). However, the number of false positives that this tool could generate might place a burden on the healthcare system—the clinical relevance of this must be considered in the context of the current standard practice for detecting anaemia in rural and underserved populations.

4.2. Pigmentation

We observed significant scleral pigmentation. Melanin, the compound responsible for visible pigmentation, has absorption in similar areas of the frequency spectrum to haemoglobin. This means that it is difficult to separate out melanin from haemoglobin using a camera with only three colour channels—the brown pigmentation is not easily distinguished from dark areas with red blood vessels. We suggest that this may be a potential cause of the poor performance demonstrated here.

However, even when participants with visible pigmentation were excluded from analysis, results remained significantly lower than following the same protocol in preschool-aged children in Ghana.

This lack of performance may be because of sampling effects (a limited range of haemoglobin concentrations). It may also be due to other physiological changes in the eye, or because not all pigmentation is being detected in this study—this research only visually judged pigmentation on the sclera. For example, this pigmentation is present in the conjunctival layer and so likely to be present also on the palpebral conjunctiva of the lower eyelid. This may explain the reduced performance on analysis of the lower eyelid, when compared to the preschool-aged children in Ghana. However, against the red background of the dense capillary bed in the eyelid, this pigmentation is difficult to detect by eye.

It is possible that CNN-based approaches may be able to identify patterns in the images which allow them to effectively ignore areas containing colours unrelated to the blood haemoglobin concentration—or even to correct for a potential relationship between pigmentation and blood haemoglobin concentration. However, such an approach would come with the risks of (a) potential overfitting to the training data, and (b) introducing a ‘black box’ computation into the algorithm, which would make it harder to explain and understand the limitations of the algorithm.

4.3. Wider implications

In producing a screening tool with the potential to reach underserved populations, we note that there is a risk of amplifying existing health inequalities—we demonstrated that results in one population did not transfer to a new population. These populations differed in ethnicity, lifestyle, age, and sex ratio. We were unable to attribute our results to a specific feature of the population. As a result, we suggest that open-access datasets and research studies in this field should measure and disclose enough participant metadata to make it possible for the reader to determine which populations the results might apply to.

Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

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Conflict of interest

None declared.

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