Antenatal haemoglobinopathy screening: Patterns within a large obstetric service. Working towards a standard of care

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Abstract

Background: Antenatal screening can predict clinically significant haemoglobinopathies, however in Australia, practices are not standardised and are evolving as the population becomes more ethnically diverse. This study describes antenatal screening practices in a large Australian laboratory/antenatal service.

Methods: Data were collected retrospectively on consecutive antenatal haemoglobin electrophoresis over 16 months and correlated with obstetric data, obtained from the local obstetric database.

Results: 462 patients were included, with an average gestation of 25.8 weeks. 'Pregnancy' was the most common stated indication, with absent indication/clinical information in 8%. Gestational age was documented in 54%. In 15%, no contact details of the referrer were documented and partner screening was traceable in only 25 cases (5.4%). In 82% of cases, no abnormalities were detected. Beta thalassemia trait was the most common positive result. Only 52% of patients had recent iron studies. The mean haemoglobin was 111.6 g/L and mean cell volume was 80.5 fl at the time of testing. Ethnicity was documented on the request form in 3%. After Australasia, the most common ethnicity of patients was South East Asia and the Middle East.

Conclusion: Referral patterns in our health service are diverse and reflect our changing population and care practices. Detailed guidelines are required and we propose a comprehensive algorithm for general use where selective screening is practiced within an Australasian population or one with similar demographics.

Keywords

Haemoglobinopathies, thalassemia, anaemia, genetic diseases, pregnancy

Background

Haemoglobinopathies are a group of recessively inherited blood disorders characterised by either the reduced synthesis of normal globin chains or the synthesis of a structurally abnormal globin chain.¹ They are the most common single gene disorder worldwide, leading to significant morbidity and mortality.² Haemoglobinopathies occur more frequently in populations originating from Southern Europe, Middle East, Africa, Asian, Pacific and Indian.² In Australia, the carrier frequency is increasing with changing migration patterns and intermarriage between ethnic groups making those at risk less obvious.³

There is a wide disease phenotype spectrum from death-in-utero with Haemoglobin Barts Hydrops Fetalis, severe lifelong transfusion-dependent anaemia in homozygotes to asymptomatic carriers.^{1,4} Additionally, the genetics of these diseases is complex, the at-risk ethnicities are diverse and the clinical severity in offspring can be difficult to predict.^{1,5} Appropriate testing and antenatal screening for these disorders are therefore difficult but important, and no standard protocol currently exists.^{4,6,7}

From the laboratory perspective, barriers exist to provide an accurate, timely and cost-effective service for the antenatal detection of haemoglobinopathies. These include late presentation and booking of antenatal patients, availability of the patient's partner for concurrent screening, lack of information regarding ethnicity and obstetric history and difficulties in communication between the clinical groups involved.⁸

Aims

To describe the use of haemoglobin electrophoresis (HbEPG) and high-performance liquid chromatography (HPLC) to screen antenatal patients for haemoglobinopathies in a large obstetric service in an area with high migration. We aimed to describe referral patterns, positive results, patient characteristics and clinical information available to the laboratory in order to evaluate barriers and generate an antenatal haemoglobinopathy screening algorithm.

Materials and methods

Ethics approval was granted (HREC 10/066). In this retrospective observational audit, the following data were collected from all requests for antenatal screening between July 2009 and November 2010: clinical information, demographics, HbEPG and HPLC results, full blood count (FBC) and iron studies. Mean cell volume (MCV) was used to identify microcytic patients at higher risk of haemoglobinopathy carriage. Mean cell haemoglobin (MCH) was included in the FBC, but not used to stratify risk by the laboratory at the time of this audit. Further clinical data were obtained from the routine obstetric outcome database (OBSTETRIX). 'Place of birth' was a proxy marker of ethnicity. Screening turnaround times were calculated for the designated period.

HPLC was performed using HPLC (Biorad Variant II), and where indicated alkaline and acid gel electrophoresis (Helena laboratories) and HbH preparation (Brilliant Cresyl Blue). Results were correlated with red cell parameters, blood film and iron studies. DNA analysis was not included in this study as it was an audit of the existing screening practice and is not routinely accessed. Eligible patients were currently pregnant, had recently miscarried/suffered stillbirth, presented for family planning or fertility testing, or were referred by an obstetrician or midwife.

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Results

Four hundred and sixty-two request forms (453 patients) were eligible of 805 samples that were processed in the specified time period. Multiple analyses were undertaken in nine patients due to coexistence of iron deficiency at initial assessment. Antenatal requests accounted for 57% of the total workload. The mean turnaround time from collection to result was 3.6 days.

Table 1 indicates the patient characteristics recorded and derived after database linkage including age, gestation at testing and presentation to antenatal care, haemoglobin (Hb) and MCV.

The majority of referrals came from local major obstetric services (59%) followed by tertiary public obstetric services (30%), and smaller numbers from preconception services (2%) private obstetric practice (8%) and general practice (1%).

Stated reasons for testing correlated with patient selection with 'pregnancy' and 'anaemia' being the sole descriptor in almost three quarters of cases (53% and 18%, respectively). In 9% of cases, the indication was either a known personal or partner history of haemoglobinopathy or family history of haemoglobinopathy, and a further 9% were done as a result of fetal loss, infertility or recurrent miscarriage. 'Ethnicity' was the indication in only 2% of cases and no clinical information was provided for 8%.

Other notably absent information on the request form included gestational age (46%), ethnicity (97%) and clinician contact details (15%). Traceable partner screening was conducted in 25 cases resulting in one partner of a known beta (β) thalassaemia trait carrier being diagnosed with β thalassaemia trait.

Where patients were found to be iron deficient, testing was repeated at the requesting clinician's discretion; a universal recommendation is issued with results to repeat testing once the patient is iron replete. Results were included if repeat testing was done in the specified audit period.

Ethnicity/place of birth were determined after linkage in 76% (Figure 1). Patients were predominantly Australasian (41%), followed by South East Asian and Middle Eastern (13% each), Asian (excluding South East Asia) (12%) and originating from the Indian subcontinent (11%). Of Australasian patients, screening was requested despite a normal MCV in 45% and normal MCV and Hb in 14%.

On screening, 82% had no abnormalities detected. Of the patients with a haemoglobinopathy, 38% were β thalassaemia trait, 22% were α thalassaemia trait and 22% were Hb E trait (Table 2). All patients had a recent FBC and iron studies were available in 52%. Of those with iron studies, 38% were iron deficient (ferritin < 20 μ g/L).

Discussion

Clinically significant haemoglobinopathies are an important public health issue where rapid, simple and inexpensive antenatal screening tests can lead to disease prevention.^{5,7} In Australia, there are increasing numbers of people with high carrier frequency ethnic backgrounds,^{6,9}

and antenatal screening is not standardised and practices are evolving as our population diversifies. Testing is frequently ad hoc, reflecting a lack of universal agreement and understanding of screening processes amongst clinicians. This study is the first detailed description of current antenatal haemoglobinopathy screening practices in Australia.

A wide variety of clinicians are involved in the detection and management of haemoglobinopathies.³ General practitioners, obstetricians, midwives, clinical and laboratory haematologists and medical geneticists are all involved in antenatal care and some have limited experience with managing haemoglobinopathy risk. In antenatal health services where *universal* screening is not appropriately based on carrier prevalence, selective screening needs to be designed to predict the great majority of at-risk pregnancies.¹ This study has illuminated concerns pertaining to the timing of screening, inadequate documentation on request forms and the appropriateness of current screening patterns. It has highlighted that the health service would benefit from specific, clear and detailed guidelines for selective screening.

1. Timing of screening

Late carrier detection can lead to adverse outcomes in offspring. Ideally, haemoglobinopathy screening would occur preconception or in the first 10 weeks of gestation in high-risk couples^{1,10} to enable completion of laboratory assays, genetic counseling and DNA testing, with the option of pregnancy termination before 12 weeks gestation where acceptable.⁴ The mean assay turnaround time in our study allowed timely communication with clinicians. The advanced gestation at time of screening (median 15 weeks) indicates that current practices identify at-risk women too late. Whilst there is variation in the clinical practice of referrers, the authors acknowledge that in many cases this reflects a broader problem of late presentation to Public Obstetric Services and the less intensive use of antenatal services by women from ethnic minorities.¹⁰ Our data support this assertion and echoes the concern that this may result in missed haemoglobinopathy diagnoses within the community.^{2,11,12} This phenomenon is likely to relate to language, social and cultural barriers, whose further exploration is beyond this study's scope, but important to acknowledge in order to improve migrant health.^{1,12} Despite these complexities, the need for clear guidelines pertaining to the timing of testing must exist for the benefit of those caring for antenatal patients in order to safely identify the great majority of positive cases. Where pregnancy termination is not acceptable, premarriage or preconceptional screening can provide couples with the necessary genetic information for the purposes of family planning.

2. Patient selection for screening

An important question that the study addressed was whether patients were appropriately selected for haemoglobinopathy screening. Effective screening is designed by identifying markers that best predict disease^{1,13} – most selective screening programs distinguish according to ethnicity and red cell indices.^{1,3,10,13–17}

Table 1. Patient characteristics.

Patient characteristic	Number of women	Median	Mean
Age at time of testing (years)	462	31.2	29.8
Gestation at presentation to antenatal care (weeks)	399	11	12.2
Gestation at time of testing (weeks)	249	15	25.8
Haemoglobin (115–165 g/L)	462	112	111.6
MCV (80–100 fl)	462	81	81

Note: MCV, mean cell volume.

Figure 1. Ethnicity according to place of birth from linkage.

Table 2. Haemoglobinopathy screening results (HbEPG and HPLC).

Result	Number
No abnormality	378
α Thalassemia trait	19
HbH disease	1
β Thalassemia trait	32
HbS trait	5
HbS disease	1
HbE trait	19
HbE disease	2
HbD trait	2
Equivocal result	3

Note: HbEPG: haemoglobin electrophoresis; HPLC, high-performance liquid chromatography.

a. Red cell indices

A normal MCV (≥ 80 fl) excludes most haemoglobinopathy carriers, however sickle cell trait, some α thalassaemia trait and rare β thalassaemia trait can be present with a normal MCV.¹⁴ Within those patients screened who had a normal MCV (≥ 80 fl, 246 patients), one β thalassaemia trait, one Hb D trait and one Sickle trait were detected.

The average MCV of patients studied (81 fl) is within the laboratory reference range indicating that many screening tests were performed on normocytic patients. This begs the question whether in cases where no indication for screening was documented (the majority), was this appropriate screening? Cross-database correlation confirmed that some of these patients originated from areas with high carrier rates of thalassaemia, although this information was not available to the laboratory at the time of testing. This either highlights a problem in documentation of clinical information on request forms in patients

who have characteristics that would qualify for selective screening or suggests that large numbers of women are being screened unnecessarily. The ideal MCV cutoff is not established.

Fifty-eight percent of patients in our study had anaemia, however only 34% of these were microcytic. Again, in the absence of documented additional risk factors, this is likely to represent inappropriate screening patterns in a subset of patients. Inappropriate screening unnecessarily utilises the health budget and laboratory personnel.

b. Ethnicity

According to 2011 Australian Census Data, the largest group of overseas-born Australian residents is from the United Kingdom and New Zealand (4.2% and 2.2%, respectively), followed by China (1.8%), India (1.5%), Vietnam (0.9%) and Italy (0.9%).¹⁸ With the exception of the United Kingdom and New Zealand, each of these areas is associated with a higher rate of thalassaemia carrier status.^{2,17} In Australia, data denoting specific carrier prevalence rates are not available, but estimated according to prevalence in the country of birth. Most countries now have an uneven distribution of carriers because their populations include different ethnic groups that have been colocated.¹¹ This study used 'place of birth' as a proxy marker of ethnicity, collected at booking for obstetric care rather than specifically for haemoglobinopathy screening. We acknowledge that it may not be accurate to assume ethnicity from country of birth or surname and that true ethnicity is difficult to ascertain, given generations of immigration. Ethnicity was poorly documented by clinicians who requested haemoglobinopathy screening and was documented in 11 cases only. Ethnicity aids in interpretation of the screening tests and partner haemoglobinopathy assessment, and hence this information is necessary for risk assessment by the laboratory.^{1,6,10} There is a clear need to document ethnicity or any known family history of haemoglobinopathy. Our laboratory hopes to assist with the interpretation of screening tests because the results are often not definitive and clinical staff are not trained in this area, however clinical information, such as ethnicity and iron status, must be available to the laboratory. Subsequent to this audit, we have instituted a multidisciplinary meeting

with the laboratory scientist, haematologist, clinical geneticist and obstetric genetic counselor, to discuss difficult results, assess risk to specific couples and optimise patient care.

Additional findings

The most common haemoglobinopathy detected in screening was β Thalassaemia trait reflective of the prevalence of mutations affecting this gene in our community.⁶

The Australian Genetics Handbook: Genetics in Family Medicine recommends that the partners of pregnant women from high-risk ethnic backgrounds be screened contemporaneously.⁵ Partner details and evidence of partner screening were available in only 25 cases of our audit reflecting another deficiency in clinical documentation. Partners

may have been screened at another laboratory by the requesting clinician; however, the lack of universal traceability and information provision to the testing scientists and haematologists impairs clinically relevant interpretation of results. Partner results aid in de-escalating or escalating DNA testing – scenarios where time frames are critical. It is acknowledged that nonpaternity may affect willingness to submit screening samples.¹² In the absence of curative therapies, counseling at-risk couples to enable informed reproductive decision making is important.^{7,16} We found that a relatively small number of cases in our audit were identified as part of preconceptional testing. Due to lack of documentation, this number may be underestimated by our analysis.

In haemoglobinopathy screening, ferritin is ordered to identify iron deficiency, which is the main differential diagnosis for microcytic anaemia.^{1,19,22} It is considered good practice to obtain the FBC and ferritin

Figure 2. Antenatal haemoglobinopathy selective screening algorithm.

in all women presenting for antenatal care and provision of these results to the screening laboratory is important for result interpretation.³

Limitations

This retrospective study relied on accurate collection of data by other parties. Linkage of patient data with offsite databases has resulted in some missing data, which may cause bias in the analysis of certain results. The rate of alpha thalassaemia trait in this audit may be underestimated as HbH staining preparations have a low sensitivity for one gene deletions of alpha thalassaemia. Eighty-four positive cases were identified, representing 18% of all tests performed and we are unable to determine whether this is an appropriate detection rate. Identifying affected offspring born to mothers who were and were not screened was beyond the scope of our study, although would be of future interest.

Antenatal Haemoglobinopathy Screening Algorithm

Wide circulation of a set of detailed and comprehensive clinical guidelines amongst staff referring patients for haemoglobinopathy screening would certainly help to standardise referral patterns, improve ordering practices and result interpretation, and hence optimise patient outcomes. This would include general practitioners, obstetricians, midwives, junior medical staff and haematologists for whom the algorithm should function as a clinical aid to consultation and review. Such a document would maintain utility in areas of Australia where selective screening is practiced.

Equally important to referring practices is the laboratory's timely and clear communication of results to the referrer. In some jurisdictions, the laboratory may initiate genetic testing, whereas more commonly a further sample must be drawn. Ultimately, fetal risk is determined by maternal and paternal genotype – ideally assessed by an experienced haematology service accessing all clinical and blood results of the couple.

The algorithm shown in Figure 2 is based on review of the literature and the results of this study placing an emphasis on timely patient and partner screening, appropriate test sets to order and risk factor documentation.

It should be acknowledged that the timing of partner screening remains controversial though results may be crucial in identifying at-risk pregnancies. Earlier testing carries the advantage of greater diagnostic certainty but at a cost of potentially unnecessary investigations. To the authors' knowledge, there is currently no published data addressing this quandary. In addition, not all women who carry a haemoglobinopathy trait may be identified using selective screening as not all structural variants are associated with microcytosis or anaemia and are not identified as being from 'at-risk' patients. The corollary of this is that women should not be cleared of haemoglobinopathy carriage unless formal testing is undertaken.

Conclusion

The pervasive theme demonstrated is a need for early testing and the provision of relevant clinical information at the point of request for haemoglobinopathy screening. All requests should include accurate information on ethnicity (not just country of birth), partner demographics and ethnicity, ferritin, other risk factors such as family carriers, gestational age and a specific indication for testing. This could be achieved by education targeting those who are likely to screen antenatal patients such as general practitioners, obstetricians, junior medical officers and midwifery staff. In our service, combined meetings with both clinical and laboratory staff have proven useful for the clinical correlation and interpretation of results, particularly those which

are complex or borderline and profiling the risk of specific couples, to guide genetic testing. Such multidisciplinary meetings result in improved patient outcomes, medical collaboration and practitioner education and understanding.

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Ethical approval

This research was approved by the Prince of Wales Hospital Ethics Committee and complied with all standards set out by the committee (HREC 10/066).

Guarantor

GKG

Contributorship

OL was responsible for data collection, analysis and writing the paper. GKG was the supervisor for this project and provided methodological guidance, expert opinion and edited the article.

References

1. Bain BJ. Haemoglobinopathy diagnosis: algorithms, lessons and pitfalls. *Blood Rev* 2011; 25: 205–213.
2. Henderson S, Timbs A, McCarthy J, et al. Incidence of haemoglobinopathies in various populations—the impact of immigration. *Clin Biochem* 2009; 42: 1745–1756.
3. The Australian Handbook for General Practitioners. Genetics in Family Medicine. Haemoglobinopathies 2007. <http://www.gpgenetics.edu.au>.
4. Cao A and Rosatelli MC. Screening and prenatal diagnosis of the haemoglobinopathies. *Baillieres Clin Haem* 1993; 6: 263–286.
5. Chui DHK, Cunningham MJ, Luo H, et al. Screening and counseling for thalassemia. *Blood* 2006; 107: 1735–1737.
6. Bowden DK. Screening for thalassemia. *Aust Prescr* 2001; 24: 120–123.
7. Modell B, Petrou M, Layton M, et al. Audit of prenatal diagnosis in the United Kingdom: the first 20 years. *BMJ* 1997; 315: 779.
8. Leung TN, Lau TK and Chung TKh. Thalassemia screening in pregnancy. *Curr Opin Obstet Gyn* 2005; 17: 129–134.
9. Argent E, Emden P, Monagle P, et al. Australian Paediatric Surveillance Unit study of haemoglobinopathies in Australian children. *J Paediatr Health* 2012; 48: 356–360.
10. Shakespeare J. Antenatal haemoglobinopathy screening. *BMJ* 2010; 341: 897.
11. Aspinall PJ, Dyson SM and Anionwu EN. The feasibility of using ethnicity as a primary tool for antenatal selective screening for sickle cell disorders: pointers from the research evidence. *Soc Sci Med* 2003; 56: 285–297.
12. Reed K. “He’s the dad isn’t he?” Gender, race and the politics of prenatal screening. *Ethnic Health* 2011; 16: 327–341.
13. Sanchaisuriya K, Fucharoen S, Fucharoen G, et al. A reliable screening protocol for thalassemia and haemoglobinopathies in pregnancy. *Am J Clin Path* 2005; 123: 113–118.
14. Sriachitayakul S, Maneerat J, Sa-nguansemsri T, et al. Sensitivity and specificity of mean corpuscular volume testing for screening for α thalassemia-1 and β thalassemia traits. *J Obstet Gynaecol Res* 2005; 31: 198–201.

15. Leahy TR, Gorman WA, Murphy C, et al. Haemoglobinopathy screening in a maternity hospital. *Irish Med J* 2006; 99: 184–185.
16. Norton ME. Genetic screening and counselling. *Curr Opin Obstet Gynecol* 2008; 20: 157–163.
17. Bain BJ and Chapman C. A survey of current United Kingdom practice for antenatal screening for inherited disorders of globin chain synthesis. *J Clin Path* 1998; 51: 382–389.
18. Australian Bureau of Statistics. Australian Census Data 2011 [cited 2013]. www.abs.gov.au/census (2013, June 2011).
19. Passarello C, Giambona A, Cannata MV, et al. Iron deficiency does not compromise the diagnosis of high HbA2 beta thalassemia trait. *Haematologica* 2012; 97: 472–473.
20. Cao A and Galanello MD. Effect of consanguinity on screening for thalassemia. *New Engl J Med* 2002; 347: 1200–1202.
21. Al-Aama J. Attitudes towards mandatory national premarital screening for hereditary haemolytic disorders. *Health Policy* 2010; 97: 32–37.
22. Harthoorn-Lasthuizen EJ, Lindemans J and Langenhuijsen MM. Influence of iron deficiency anaemia on haemoglobin A2 levels: possible consequences for beta-thalassemia screening. *Scand J Clin Lab Inv* 1999; 59: 65–70.