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Antenatal screening for haemoglobinopathies: current status, barriers and ethics

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Summary

Sickle cell disease (SCD) and thalassaemia are genetic disorders that are caused by errors in the genes for haemoglobin and are some of the most common significant genetic disorders in the world, resulting in significant morbidity and mortality. Great disparities exist in the outcome of these conditions between resource- rich and resource-poor nations. Antenatal screening for these disorders aims to provide couples with information about their reproductive risk and enable them to make informed reproductive choices; ultimately reducing the likelihood of children being born with these conditions. This review provides an overview of the current status of antenatal, pre-marital and population screening of SCD and thalassaemia in countries with both high-and low prevalence of these conditions, methods of screening in use, and discusses some of the pitfalls, ethical issues and controversies surrounding antenatal screening. It also discusses outcomes of some screening programmes and recognises the need for the establishment of antenatal screening in areas where their prevalence is highest; namely sub-Saharan Africa and India.

Keywords: sickle cell disease, thalassaemia, prenatal diagnosis.

Introduction

Antenatal screening for genetic disorders, such as Sickle cell disease (SCD), β-thalassaemia and α-thalassaemia, aims to reduce the burden of these diseases by offering information to individuals with a high likelihood of giving birth to affected babies and giving parents more choice regarding their reproductive decisions, including prenatal genetic diagnosis and termination of pregnancy. In most cases, screening is offered to pregnant women during early gestation, allowing parents to make an informed decision about their reproductive options. However, in countries where medical termination of affected pregnancies is not permitted or acceptable, screening is offered prior to conception, often during the premarital stage at which couples choose their reproductive partners. In some countries or regions with high prevalence of disease or carrier rates, screening is offered to children of school age, long before individuals reach reproductive capacity, with the aim of allowing 'at risk' individuals to make an informed choice of their future reproductive partners. In contrast, newborn screening identifies those children born with the condition in order to offer treatment and prevent early mortality. In this review, we evaluate the current antenatal, pre-marital/pre-conceptual and population-based screening programmes for haemoglobin disorders globally and discuss the operational and ethical challenges faced by these programmes. For comprehensive reviews of newborn screening for SCD and thalassaemia globally, the reader is referred to three excellent recent articles (Lobitz *et al*, 2018) (Therrell & Padilla, 2018) (Therrell *et al*, 2015).

Sickle cell disease and thalassaemia are genetic disorders that are caused by errors in the genes for haemoglobin and are the most common significant genetic disorders in the world, resulting in very high rates of mortality and morbidity and causing significant health and economic burden to individuals and economies (Piel *et al*, 2017). It is estimated that 300,000 children are born with SCD each year, 50% of whom are born in just three countries- the Democratic Republic of Congo, India and Nigeria (Piel *et al*, 2013).The majority of the rest are born in other parts of Africa; although the slave trade and population movement has resulted in affected children born in many European countries and the Americas (Piel *et al*, 2014; Lindenau *et al*, 2016). Mortality in Africa is the highest and limited data indicates an under-five years of age mortality of 7·3/100 patients/year in a hospital cohort in Tanzania (Makani *et al*, 2011; Wastnedge *et al*, 2018) and is likely to be significantly higher in rural areas in Africa, with childhood mortality estimated between 50–90% (Grosse *et al*, 2011). The β-thalassaemias account for about 60,000 births a year, predominantly in South East Asia, India, Central America, Middle East and the Mediterranean countries (Modell & Darlison, 2008; Weatherall, 2010). Without regular blood

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transfusions and iron chelation therapy, mortality is high in childhood. Despite this, the incidence of these conditions is set to rise as a consequence of improvements in childhood survival through general public health measures in high prevalence low- and middle-income countries (Weatherall, 2010). The World Health Organisation (WHO) has recommended development of screening programmes, epidemiological surveillance programmes, disease control strategies, treatment protocols and healthcare personnel training in high prevalence countries, recognising its significant adverse effects on childhood health and mortality WHO, 2007). The decision to conduct antenatal or premarital screening for either SCD, thalassaemia, or both, is determined mostly by local disease prevalence and overall cost-effectiveness of running largescale programmes. In countries where both diseases are prevalent, there is good argument to ensure both conditions are screened antenatally (Koren *et al*, 2009). In England, antenatal screening detects couples at risk of having a baby affected by both thalassaemia major and SCD, but newborn screening (NBS) only aims at detecting variant haemoglobins that can cause sickling disorders. The linked antenatal and neonatal programme in England enables parents to be prepared to receive a positive NBS result and allows further review of the antenatal screening results before and after the affected baby is born to check that the results are congruent.

Global prevalence of antenatal or premarital screening for haemoglobinopathy

Screening for thalassaemia

Antenatal screening for thalassaemia has been available in many countries for a number of years where high disease prevalence and carrier rates have rendered such programmes cost-effective. Most programmes offer screening to individuals on a voluntary basis; mostly supported with counselling services and reasonably easy access to prenatal diagnosis and medical termination of pregnancy, if requested (Cousens *et al*, 2010). Universal antenatal thalassaemia screening programmes were established in Greece and Cyprus as early as 1973 (Angastiniotis & Hadjiminas, 1981; Theodoridou *et al*, 2018). Many other countries with a high prevalence of thalassaemia have gradually caught up. A national programme of mandatory pre-marital thalassaemia screening in Iran was established in 1997 (Samavat & Modell, 2004). Saudi Arabia and Turkey established their universal programmes of thalassaemia screening in the early 2000's (Canatan & Delibas, 2016, Al Sulaiman *et al*, 2008), although it is useful to note that the main emphasis of many such programmes is on premarital screening to inform choice of reproductive partner. Additionally, pre-marital, pre-conceptual or antenatal screening for thalassaemia is available in Palestine, Israel, Madagascar and Italy, (Cousens *et al.*, 2010) among others. Many Asian countries with high prevalence of β- and α-thalassaemia also have well-established antenatal screening programmes; notably the Maldives (since 1992; mandatory since 2012), Sri Lanka (national pre-marital and population screening started in 2006), Thailand (since 1997), Singapore (since 1997), Malaysia, Philippines and Hong Kong (Goonasekera *et al*, 2018). Screening for thalassaemia, SCD and other β-haemoglobinopathies is available in certain pockets of high prevalence in India, targeting tribal groups in Central and Southern India, and in the States of Gujarat and West Bengal. Here, the focus is often on antenatal screening and cascade screening of family members of affected individuals, owing to the stigma associated with having a haemoglobinopathy carrier status before marriage (Colah *et al*, 2008; Verma *et al*, 2011). Antenatal screening for thalassaemia in high-income, low prevalence countries, such as the UK, Canada and Australia were established at the turn of the 21st century (Goonasekera *et al*, 2018). England is unique in this respect and is the only country where a linked programme of antenatal and postnatal thalassaemia and SCD screening is in existence. (Locock & Kai, 2008).

Screening for sickle cell disease

Antenatal screening for SCD is not as widely available as that for thalassaemia. Some high-income countries offer antenatal screening for SCD - notably the UK, Canada and Australia. Region-specific or isolated pilots for antenatal screening is available in some countries where SCD is endemic, such as that in India (Bhukhanvala *et al*, 2013), Cuba (Aguila *et al*, 2008) and Northern Israel (Koren *et al*, 2009). Some African countries, such as Ghana, Burkina Faso, Benin, Congo and Nigeria, have reported on isolated, regional NBS pilots, but large-scale national haemoglobinopathy screening programmes are lacking (Hsu *et al*, 2018).

Antenatal screening methods

Antenatal (or population-based, pre-marital or pre-conceptual) haemoglobinopathy screening methods include laboratory and field-based blood tests as well as information regarding family origin. Choice of methods used depends on individual national policies and is determined by cost of the tests, the type of prevalent haemoglobin disorders, ethnic diversity of the population screened and infrastructure available to support the screening programmes (Goonasekera *et al*, 2018).

Laboratory-based blood tests include a full blood count (FBC) with pre-determined cut-offs for actionable red cell indices, such as mean corpuscular haemoglobin (MCH) or mean corpuscular volume (MCV), haemoglobin separation and HbA_2 quantification methods, including gel electrophoresis, high performance liquid chromatography (HPLC), capillary electrophoresis and isoelectric focussing. Additionally, examination of blood smears for HbH inclusion bodies is undertaken in some areas with high prevalence of α^0thalassaemia carriers. Over 200 thalassaemia mutations

haven been described, and DNA-based tests chosen for screening programmes reflect local gene mutation profiles, cost of tests, expertise of personnel and scalability of assays. Hence a large number of techniques, such as Gap-Polymerase Chain Reaction (PCR), Southern blot, Amplification-refractory Mutation System (ARMS), Reverse Dot Blot Analysis (RDB), Single Strand Conformation Polymorphism (SSCP), denaturing HPLC, Multiplex Ligation-dependent Probe Amplification (MLPA) and sequencing are in use (Cao & Kan, 2013). More recently, high-throughput next generation sequencing analyses have been used for population screening of thalassaemias in Southern China (Zhao *et al*, 2019).

Field-based blood tests are generally cheaper than laboratory tests and rely on physical properties of thalassaemic red cells. A particularly good example is the Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) that relies on the reduced osmotic fragility of microcytic thalassaemic red cells. The dichlorophenolindophenol (DCIP) dye test can identify unstable Hb, such as the variant HbE. NESTROFT and DCIP are used in combination in South East Asian screening programmes in low-resource countries, such as Thailand and India, where both HbE and β-thalassaemia are prevalent (Fucharoen *et al*, 2004; Fucharoen & Winichagoon, 2011; Mohapatra *et al*, 2016). Samples that test positive on the field tests can then be subjected to more specific testing, such as DNA diagnosis (in α-thalassaemia) or haemoglobin separation techniques. See Table I for the various screening methods available globally.

In populations where iron deficiency anaemia (IDA) is highly prevalent, initial screening with red cell indices and HbA_2% may falsely identify iron-deficient patients as having β-thalassaemia trait (BTT) or may miss those individuals with both IDA and BTT in whom iron deficiency has caused a reduction in the HbA_2%. Several formulae using red cell counts, MCV and MCH (known collectively as Discriminant Function) have been proposed to improve detection rates of true carriers (England & Fraser, 1979). Several of these Discriminant Functions have poor validity in the context of pregnancy, but a combination of the MCV, Shine and Lal index $[(MCV)^2 \times MCH/100,)]$ (Shine & Lal, 1977) and recall of patients with the Shine and Lal index < 1530 for confirmatory testing has been successfully used in resource-poor settings (Yeo *et al*, 1995). A recent report from Israel confirms the utility of a computational program using simple red cell indices to predict IDA and BTT (Roth *et al*, 2018). This formula had a high sensitivity, similar to the Shine and Lal Index, and was validated in a large diverse population, including anaemic and pregnant women. This could be used as a first line screening tool in populations with high prevalence of both conditions (Roth *et al*, 2018).

Outcomes of antenatal screening programmes

In countries with high population carrier rates of haemoglobin mutations, particularly in those where consanguineous marriages are common, the resultant high numbers of affected births pose a large burden on the national economy. It is hard to overstate the extent of this global public health issue. Haemoglobin disorders are highly prevalent in 71% of the world's 229 countries. This is where 89% of the world's babies are born every year. This was recognised by WHO, who urged such countries to implement national prevention and treatment programmes WHO, 2007). However, the guidelines also stipulated that screening should not be mandatory and only undertaken after informed consent. Based on gene frequency estimates and WHO guidance, 9 million carriers and their partners should be offered genetic counselling annually; 113 million women who become pregnant annually should be offered haemoglobinopathy screening and 1·88 million pregnant women should be offered prenatal diagnosis (Modell & Darlison, 2008).

However, haemoglobinopathy screening strategies are still not universally established, and significantly fewer pregnant women and couples are offered such services. The success of large-scale population-based programmes depends on political and social will and robust education initiatives for the public as well as healthcare workers. Locally-relevant evidence on gene frequency, mutation spectrum in case of thalassaemias, carrier states and pertinent, cost-effective tests should inform local screening policy, and adequate logistical support should be made available to execute such programmes, including the offer of prenatal diagnosis in at-risk couples. Using such initiatives, several examples of well-established national haemoglobinopathy screening programmes are available. Perhaps the earliest and most notable example of a screening programme in β-thalassaemia comes from Cyprus (Angastiniotis & Hadjiminas, 1981), where mandatory premarital screening, prenatal diagnosis in high risk couples and development of thalassaemia specialist centres providing high quality, state-funded treatment was established in the 70's. This has resulted in almost no affected births with this condition in the country within three decades of its inception. Several other programmes are worthy of mention and mostly pertain to β-thalassaemia screening, leading eventually to the reduction of the birth of affected children within those programmes. Currently, antenatal screening programmes have not been established in countries with a high prevalence of SCD, although it undoubtedly deserves to be a high public health priority in endemic nations.

See Table II for a list of countries that have demonstrated reduction in affected births since the establishment of screening programmes.

Prenatal diagnosis

An essential adjunct to any successful antenatal screening programme is the availability of prenatal diagnosis (PND) in a pregnant woman who is at a high risk of giving birth to a baby affected with a major haemoglobin disorder. This

Table I. Antenatal Haemoglobinopathy Screening methods currently in use. in some countries. This is not an exhaustive list of countries where antenatal screening is available, but representative programmes covering all continents are included.

Country	Universal or targeted	Type of screening	Primary methods used	Comments	Reference
England	Universal	Antenatal	HP areas: FBC, HPLC, CE, DNA for α-thalassaemia and FOQ. LP areas: FOQ followed by HP protocol if high risk	Part of a linked antenatal and neonatal screening programme, voluntary	NHS Sickle Cell and Thalassaemia Screening Programme (2017)
Wales	Universal	Antenatal	English LP protocol	Voluntary	Public Health Wales (2019)
Scotland	Universal	Antenatal	English LP protocol	Voluntary	National Services Scotland (2019)
Cyprus	Universal	Antenatal, premarital and preconceptual	FBC, HE, BF	Mandatory	Angastiniotis and Hadjiminas (1981)
Greece	Universal	Premarital and antenatal	FBC, HE/HPLC, BF, Sickling test	Voluntary	Theodoridou *et al* (2018)
Italy	Regional	Antenatal, school age	FBC, HPLC	Voluntary	Amato *et al* (2014); Bianco *et al* (1985)
France	Regional	Secondary school children	FBC, HPLC, IEF, HE	Voluntary	Lena-Russo *et al* (2002)
Portugal	Regional	Premarital and antenatal	FBC, HPLC	Voluntary	Martins *et al* (1993)
Lebanon	Universal	Premarital	FBC, HE	Mandatory	Abi Saad *et al* (2014)
Palestine	Regional	Premarital	FBC, HE	Mandatory	Tarazi *et al* (2007)
Turkey	Universal	Premarital	FBC, HPLC	Voluntary	Canatan *et al* (2006)
Saudi Arabia	Universal	Premarital	FBC, HPLC	Mandatory	Alhamdan *et al* (2007)
Iraq	Regional (Kurdistan)	Premarital	FBC, HPLC	Mandatory	Al-Allawi *et al* (2015)
Iran	Universal	Premarital	FBC, HPLC	Mandatory	Samavat and Modell (2004)
Maldives	Universal	Premarital	FBC, HPLC	Mandatory	Firdous *et al* (2011)
Sri Lanka	Universal	Premarital	FBC, HPLC	Voluntary	Goonasekera *et al* (2018)
India	Regional	Premarital, preconceptual, antenatal, population, cascade	FBC, OFT, HPLC, HE	Voluntary	Colah *et al* (2008)
Thailand	Regional	Antenatal	FBC, OFT, HE, HPLC	Voluntary	Ratanasiri *et al* (2006)
Singapore	Universal	Antenatal	FBC, HPLC, HE, HbH inclusion	Voluntary	Tan (2005)
Malaysia	Universal	Antenatal, premarital, schoolchildren, cascade	FBC, HPLC, HE,	Voluntary	Lee *et al* (2019)
China	Regional	Antenatal, preconceptual	FBC, HPLC, HbH inclusions, HE, genotyping	Voluntary	Jiang *et al* (2017); Liao *et al* (2005)
Cuba	Universal	Antenatal	–	Voluntary	Aguila *et al* (2008)

BF, blood film; CE, capillary electrophoresis; FBC, full blood count; FOQ, family origin questionnaire; HbH, haemoglobin H; HE, haemoglobin electrophoresis; HP, high prevalence; HPLC, high performance liquid chromatography; IEF, isoelectric focussing; LP, low prevalence; OFT, osmotic fragility test.

Table II. National haemoglobinopathy programmes that have reported their screening outcomes.

Country/region, type of screening, mandatory or voluntary	Details	% reduction in affected births	Type of haemoglobin disorder prevented	Reference
Turkey Antenatal, voluntary	Southern Turkey Affected births/year: 272 (in 2002) to 25 (in 2013)	90%	SCD, β- and α-thalassaemia	Canatan and Delibas (2016)
North Cyprus Premarital, mandatory	Affected births/year: 18–20 (1981) to 0 (2005)	100%	β-thalassaemia	Bozkurt (2007)
Cyprus Antenatal, mandatory	Affected births/year: 52 (1974) to 0–1 (1996)	95-100%	β- and α-thalassaemia	Angastiniotis and Modell (1998)
Sardinia Antenatal, voluntary	Affected births year: 110 (1974) to 10 (2010)	90%	β-thalassaemia	Cao and Kan (2013)
Iran Premarital, mandatory	Affected births/year: 480 (1998) to 78 (2002)	83%	β-thalassaemia	Samavat and Modell (2004)
Maldives Mandatory population screening and voluntary antenatal	Affected births/year: 1222 (1993) to 20 (2001)	80%	β-thalassaemia, SCD	Firdous *et al* (2011)
Singapore Antenatal, voluntary	Affected births/year: 15–20 (1997) to 1 (2003)	90%	β-thalassaemia	Tan (2005)

SCD, sickle cell disease.

enables her to make an informed reproductive choice about her pregnancy and limits any medical complication as a result of continuation with her pregnancy. Advances in techniques to safely obtain fetal material in early gestation and availability of trained operators to obtain such samples are essential, as are the availability of molecular diagnostic methods that require very small amount of source material (Li & Yang, 2017).

Three techniques are currently in use for obtaining fetal samples:

Chorionic villus sampling (CVS) involves obtaining a small amount of placental tissue originating from the same fertilised egg as the fetus by needle biopsy and can be done at 11-12 weeks' gestation. Depending on placental position, the sample can be obtained either by a trans-abdominal or a trans-cervical route. Complications include fetal loss and is low in experienced hands (Kazal *et al*, 2018). It is vital that maternal contamination of fetal sample is avoided by careful dissection of the biopsy material to obtain the villi for DNA extraction and testing.

Amniocentesis is the best method to obtain fetal DNA at mid-trimester (15–18 weeks' gestation); a procedure where about 10 ml of amniotic fluid is obtained from the mother by needle aspiration and amniotic cells are obtained by centrifugation and are subjected to cell culture followed by DNA extraction and analysis (Wu *et al*, 2018). Although risk of maternal contamination is low by this method, the results take longer to obtain and may only be available when the woman is 20 weeks pregnant.

A third method is direct fetal blood sampling (FBS) by cordocentesis and DNA extraction and analysis or FBC and Hb electrophoresis of fetal blood in fetuses at risk of severe thalassaemias (Srivorakun *et al*, 2009). The latter is used in centres with no access to DNA diagnostic methods or where DNA testing has yielded equivocal results (Rao *et al*, 2009; Tongprasert *et al*, 2019). FBS is generally done in advanced pregnancy and is not the preferred method of PND. The risks of this procedure include fetal loss and the emotional burden of obtaining a positive result in late gestation, making the choice of termination significantly harder for the parents.

Several DNA-based techniques are in use for mutation analysis in PND samples, similar to those used in antenatal screening, including Gap-PCR, amplification-refractory mutation system (ARMS), reverse dot blot (RDB) and multiplex ligation-dependent probe amplification (MLPA), etc. Choice of molecular techniques is dependent upon local resources, expertise and mutation spectrum (Li & Yang, 2017).

Other techniques used in PND

Measurement of fetal cerebral artery velocity in pregnant women suspected of carrying a fetus with α-thalassaemia major is frequently used in conjunction with cordocentesis to determine the presence of anaemia and confirming the diagnosis when concomitant DNA-based techniques are not available. The combination of predetermined cut-offs of HbA% and raised cerebral velocity increase the diagnostic accuracy and enable decision-making regarding termination of pregnancy(Tongprasert *et al*, 2019).

Future direction in PND

Due to the potential threat of adverse fetal outcome in invasive techniques, non-invasive prenatal testing (NIPT) is under intense scrutiny. This either involves isolation of fetal cells (lymphoblasts, erythroblasts or trophoblasts) from maternal blood samples and undertaking DNA-based techniques for mutation detection or cell-free DNA analysis from maternal blood samples. The latter is already in widespread use for detection of aneuploidies (Herraiz *et al*, 2019) and has also recently been used to identify single gene defects, such as thalassaemias (Camunas-Soler *et al*, 2018; Xiong *et al*, 2018).

Challenges and pitfalls of antenatal haemoglobinopathy screening

Despite a WHO resolution urging all countries with a high prevalence of haemoglobin disorders to take action in prevention and treatment of such conditions (WHO, 2007), only a handful of nations have successfully established effective screening programmes. A number of challenges faced by nations preclude the establishment of such programmes; and this includes lack of political will, shortage of dedicated funds, diagnostic and interpretative challenges in areas with high genetic diversity and lack of well-established local policies and guidelines to inform the development of such programmes.

Infrastructure is often a challenge in developing population screening programmes, but is not the only determinant of poor-quality care (Leslie *et al*, 2017). Strong health leadership and political or social will has helped many nations to devise cost-effective screening methods. A good example of using existing infrastructure of primary care facilities to implement pre-conceptual thalassaemia screening is available in Iran (Samavat & Modell, 2004).

Societal acceptance of haemoglobinopathy as a significant disorder is also paramount to the development of successful screening programmes. Public awareness campaigns have been used successfully in Greece and Cyprus (Angastiniotis & Hadjiminas, 1981; Theodoridou *et al*, 2018) and also in the case of the Latium region of Central Italy, where school-age screening and awareness has successfully reduced the incidence of thalassaemia in a low-prevalence region with a large geographical coverage (Amato *et al*, 2014). Increased public awareness has led to the successful uptake of many programmes even where screening is not mandatory (Canatan *et al*, 2006).

The importance of genetic counselling to at-risk couples, administered in a culturally-sensitive way and preceded by locally researched acceptability surveys, cannot be overstated. Counselling is provided by genetic, midwifery, medical or rural healthcare staff. The UK has a programme of training for genetic counsellors available through governmental agencies (Department of Health, 2019) and other similar programmes are available internationally. However, it is also important to revisit counselling strategies and acceptability among target populations in order to ensure that every possible step has been taken to provide objective, non-directive and sensitive advice. Despite the existence of national screening programmes in England, research has suggested poor quality information is often imparted to women at antenatal screening, leading to poor psychological readiness of accepting screening results (Ahmed *et al*, 2005). The thalassaemia module of the UK Confidential Enquiry into Counselling for Genetic Disorders in 2000 demonstrated that antenatal screening and counselling for haemoglobin disorders were being delivered in an inadequate and inequitable manner (Modell *et al*, 2000).

There are several pitfalls in existing laboratory screening programmes. Programmes that rely on the use of red cell indices and FBC in thalassaemia carrier screening may be confounded by co-existing iron deficiency (Shukla *et al*, 2018) and by cases of 'silent'β- or α-thalassaemia, where MCV and MCH are not low (Lo, 2017). The use of phenotypic screens, such as osmotic fragility test, to determine the presence of β- or α-thalassaemia trait may be confounded by co-inheritance of other conditions, such as glucose-6-phosphate dehydrogenase deficiency or South-East Asian Ovalocytosis that reduce the detection rates of thalassaemia heterozygotes to below 70% (Penman *et al*, 2015). Lack of robust genetic techniques for carrier detection may result in missed diagnoses, particularly if the individual presents with a rare mutation, or the quality of the assay was poor but inadequate quality assurance procedures leads to the release of an incorrect report. This is often the case in ad-hoc population screening obtained in private laboratories by citizens in resource-poor countries where lack of information about disease prevalence and inaccurate screening results may lead to incorrect reproductive choices with devastating consequences (Aderotoye-Oni *et al*, 2018). All techniques used for abnormal haemoglobin detection in carrier screening have their own limitations. It is therefore paramount that screening programmes acknowledge these limitations and put systems in place to reduce errors (Barrett *et al*, 2017).

Ethical aspects of antenatal haemoglobinopathy screening

A number of ethical challenges are posed by antenatal screening of genetic disorders and several international statutes govern practice to protect rights of the unborn child (WHO, 1998). Due to variation in religious, cultural and moral status among societies, careful evaluation of local beliefs and value systems are needed before establishing an effective programme of prevention of genetic disease in a particular society. Although international guidelines recommend that screening programmes are offered to citizens in a voluntary manner (WHO, 1998), many countries have implemented mandatory screening with success and full support of its

citizens. Mandatory premarital screening and genetic counselling programmes in the Middle East were evaluated in a recent study (Saffi & Howard, 2015). The authors demonstrated that, although these mandatory programmes did not result in the reduction of at-risk marriages, they were effective in reducing the prevalence of affected births in countries providing prenatal detection and therapeutic abortion (Saffi & Howard, 2015). With increased public awareness and acceptance, several countries with strong religious beliefs against termination of pregnancies, such as Iran (Firdous *et al*, 2011), Pakistan (Ahmed *et al*, 2000) and Cyprus (Angastiniotis & Hadjiminas, 1981), successfully negotiated with religious bodies to permit termination in early gestation in fetuses with severe β-thalassaemia. Additionally, a study from Iran demonstrated that shared decision-making counselling consultations resulted in a significant drop in decisional conflicts and decisional regrets in women who had undergone termination of pregnancy for β-thalassaemia major three months after the procedure compared to those who did not (Moudi *et al*, 2018).

Non-invasive PND for genetic disorders using cell-free fetal DNA obtained from maternal blood samples poses additional ethical challenges as it is becomes increasingly available through state or privately funded programmes in recent years. Although most programmes currently focus on detection of aneuploidies and fetal anomalies, it is only a matter of time before such technologies are made available for single gene disorders. Ethical challenges include inadvertent diagnoses of maternal and fetal disease pre-disposition genes, maternal cancer and its perverse use in fetal sex selection (Hui & Bianchi, 2017). Adequate maternal counselling and robust ethical framework is therefore of paramount importance when these technologies are introduced (Chandrasekharan *et al*, 2014).

Antenatal screening in the UK

Antenatal screening for haemoglobinopathies has been available in areas of high prevalence in the UK since the 1970's. Following a survey of haematologists in England and Wales in 1978 and 1979, it was estimated that 67% were screening women antenatally, though this figure varied between areas of low (62%) and high prevalence (83%) (Davis *et al*, 1981). Implementation of the NHS plan in 2000 (Department of Health, 2000)) resulted in the emergence of a linked antenatal and neonatal haemoglobinopathy screening programme in England.

The antenatal screening programme for haemoglobinopathy in England aims to offer screening to all pregnant women by 10 weeks' gestation, offer PND by 12 weeks and perform PND by 12 weeks and 6 days (NHS Sickle Cell & Thalassaemia Screening Programme, 2017). The UK Department of Health mandates the submission of quarterly quality assurance data for key performance indicators (KPI) of the screening programme, which includes timeliness of screening tests, completion of Family Origin Questionnaire (FOQ) (ETHNOS, 2006) and a timely offer of PND to 'at-risk' women (Public Health England, 2018a). The population screening programme provides a summary of the KPI reports annually for England (Public Health England, 2019).

Antenatal screening within the UK varies according to high and low prevalence areas. NHS trusts are considered high prevalence if 2% or more of samples obtained at the first blood test during pregnancy (booking bloods) are screen-positive. Alternatively, those with less than 1% screen-positive results at booking are considered to be low prevalence (NHS Sickle Cell & Thalassaemia Screening Programme, 2017). Scotland and Wales are both low prevalence countries and use the FOQ in their antenatal screening programme. Northern Ireland does not offer antenatal haemoglobinopathy screening.

In high prevalence areas, all consenting pregnant women undergo FBC and haemoglobin electrophoresis and complete the FOQ. This effectively identifies all women with significant haemoglobinopathies. HbA2 levels of 3·5% or higher, MCH of 27 pg or lower, HbF levels of 5% or higher or identification of a significant haemoglobin variant triggers the need for biological partner testing and leads to the offer of PND if the partner is also found to have similar attributes in their screening investigation. Information regarding the use of assisted reproductive techniques, e.g. donor egg or sperm, is collected. Additionally, in women with MCH of 25 pg or lower and FOQ indicating origin from China, Southeast Asia, Cyprus, Greece, Sardinia, Turkey or unknown, undergo DNA analysis for excluding α^0thalassaemia. (NHS Sickle Cell & Thalassaemia Screening Programme, 2017). In low prevalence areas, FBC from booking antenatal bloods and FOQ are considered for haemoglobinopathy screening. Further investigative action is triggered when the FOQ indicates high risk and MCH is < 27 pg. These women are then tested along the lines of high prevalence areas (NHS Sickle Cell & Thalassaemia Screening Programme, 2017). Couples identified as 'high risk' at antenatal screening are supported by a programme of counselling, which includes face to face discussion and written information, and are offered PND and termination of pregnancy in affected pregnancies. Guidance and algorithms are available to screening teams for reference (Public Health England, 2017) (Ryan *et al*, 2010).

Current status in of haemoglobinopathy screening in England

In 2016-2017, approximately 677,500 pregnant women were screened for SCD and thalassaemia in England. Of these, 12,705 women were screen-positive (1 in 53 screened) and 751 'at-risk' couples were identified (1 in 17 screen-positives). The coverage of the antenatal screening programme was >99% nationally and screening results were available by 10 weeks' gestation. A good return of the FOQ was obtained in both low and high prevalence areas (97%). However, the

uptake of fathers' screening tests was only 64%. PND tests were performed by 12 weeks and 6 days' gestation in only 40% cases (Public Health England, 2018b). This is of concern, as delays in the offer of PND may result in mothers declining termination of affected pregnancies (Ahmed *et al*, 2006).

The aim of the screening programme in England is to ensure that couples are able to make an informed choice very early in the pregnancy to carry out PND and subsequent termination, if wanted. Decisions about continuing a pregnancy that is affected by SCD will differ to one where the pregnancy is affected by β-thalassaemia major, given the variability in outcome of the former and known natural history of the latter. There are also cultural, religious and social factors that may influence the uptake of prenatal diagnosis (Ahmed *et al*, 2006). In 2016–2017, half of the couples identified as 'at risk' had a PND test. Of the 374 cases of PND carried out, 65 fetuses were affected by SCD (which would include milder cases, e.g. HbSC disease) and 13 by β-thalassaemia major (Public Health England, 2018b). The outcome of these pregnancies is not known, but not all of them resulted in termination, and as pregnancies were continued the later PND was carried out (M. Dick, unpublished observation). Numbers are small but show that PND currently in England does not influence the birth rate of haemoglobinopathies greatly.

The number of children born with haemoglobinopathy in England is small compared to many other parts of the world. In 2016–2017, of the 667,500 babies screened for SCD, 256 babies were found to have SCD and 25 were identified as β-thalassaemia major. These numbers have declined over the past 10 years (Public Health England, 2018b). Although pre-conception screening is not available in England, it is presumed that, in time, the number of people who know their haemoglobin genotype should increase, given that NBS also identifies those with Hb- S, C, D, O and E traits. However, conferring future reproductive risk information to newborn babies identified as carriers is not a stated aim of the screening programme (Bombard *et al*, 2010). Additionally, it is not clear whether this reproductive risk information of newborn carrier babies conferred to their parents at the time of their birth is used to inform birth control practice once these babies reach adulthood.

Conclusions

Antenatal diagnosis of haemoglobin disorders has been shown to be successful in reducing birth rates of affected children in many diverse societies. Whilst several ethical and operational challenges remain, countries with the highest burden of disease are currently lacking effective antenatal or neonatal screening programmes (Therrell & Padilla, 2018). Co-operative programmes between resource-rich and -poor countries (so-called North-South collaborations) have already demonstrated success in implementing screening programmes in some African countries (Hsu *et al*, 2018). To ensure success, programmes should aim to provide high quality medical care for existing patients, ensure widespread public education, lobby for political priority and establish locally effective tests and infrastructure.

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