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CHAPTER

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CHAPTER 3: PREVENTION IN ENDEMIC AND NON-ENDEMIC IMMIGRATION COUNTRIES

Hemoglobinopathies were originally endemic only in tropic and sub-tropic areas of the Old World but in the past centuries sickle cell disease (SCD) and thalassemia have spread worldwide as a consequence of slave trade, colonization and recent migrations.

National programs consisting of information, screening and counseling have been implemented in many endemic countries in order to prevent the birth of children with severe hemoglobinopathies, while emerging countries are beginning to acknowledge the importance of starting prevention strategies focusing at population screening, genetic counseling and developing reference centers to identify healthy couples at risk (1).

Primary prevention can be prospective if offered before conception or retrospective if made available after the birth of the first affected offspring (2). Different prevention strategies are available that are differently accepted in various cultures, influenced by social and religious background, economy and politics. In some countries religion plays a major tolerant or restrictive role when prevention involves pregnancy termination, in other the strategy involves mandatory premarital screening.

In some cultures carrier screening may result in changing the candidate partner if the diagnosis is made prior marriage but if the couple at-risk still decide to marry, the options they have are to a) avoid getting pregnant, b) ask for prenatal diagnosis (PD), c) opt for a non-carrier gamete donation or d) choose to adopt. However not all these options are widely accepted and most couples at-risk would opt for PD if legally permitted or pre-implantation genetic diagnosis (PGD) (3). Some cases may even take the risk and hope for a healthy child. When newborn screening (NBS) is available, it may help these couples to plan for the best-tailored treatment and to reduce the risk of the severe symptoms in case of an affected child. It is evident that the earlier carrier detection is done, the more options are available for primary prevention (4).

Most endemic countries with long prevention experience focus on early carrier detection while non-endemic countries with large populations of immigrants at risk offer mainly NBS. Although NBS is not an efficient option for primary prevention, it allows secondary (morbidity) prevention and eventually retrospective or even prospective primary prevention for the following child if the NBS procedures are properly managed and all new born carriers are reported. This is the case in the UK and to some extent in Holland where, besides national NBS, early pregnancy screening is also offered universally or regionally.

A positive aspect in hemoglobinopathy screening is that carriers can be identified by simple, fast and relatively inexpensive hematological and biochemical tests which most laboratories can offer and afford. This is fairly unique amongst human genetic diseases.

Once couples at risk are identified or suspected, accurate molecular characterization might be required to avoid misdiagnosis that can lead to potential pitfalls in genetic counseling and prenatal diagnosis when it is offered, especially in countries where the spectrum of mutations is very heterogeneous and the conditions are widespread (5).

Below we describe the available prevention option in endemic and non-endemic countries and highlight the bottlenecks of carrier diagnostics and early screenings in Muslim Arab societies and in Oman in particular.

3.1 Prevention of severe hemoglobinopathies in different countries

Prevention of hemoglobinopathies is offered to couples that are at risk for getting a child with a severe phenotype and that, although treatable at great expenses, it is associated with heavy suffering and premature death.

The most relevant hemoglobinopathies are those caused by defects on both beta genes, such as beta-thalassemia major and sickle cell disease. Being most forms of alpha thalassemia mild due to the presence of 4 alpha genes (6), prevention is only relevant for couples who are carriers of severe alpha genotypes (usually alpha-zero deletions) that are at risk of getting a child with hydrops fetalis, or for couples with combinations of alpha zero carriers and certain alpha⁺ defects that might cause severe HbH disease. Severe hemoglobinopathy conditions for which prenatal diagnosis is generally justifiable are summarized in Table 3.1.

Table 3.1. Six categories of severe HBP states, for which genetic counseling, and possibly PD is needed. The first 3 being the most common, adapted from Traeger-Synodinos, 2013 (38).

Category of hemoglobin disorder	Most frequent causes
Thalassemia major	Severe β - and/or $\delta\beta$ -thalassemia mutations
Sickle cell disease	S/S, S/C, S/ β -thal, S/D, S/O ^{Arab} , S/Lepore, S/E etc.
Hb E/ β -thalassemia	Co-inheritance of β -thal mutations with HbE
Hb Lepore and other thal variants	Co-inheritance of β -thal and Hb variants with thal or instable phenotype
Hb Bart's Hydrops Fetalis syndrome	Homozygous α^0 -thalassemia deletions
Hb H intermediate or severe	Interaction of α^0 -thalassemia deletions with severe nondeletion α -thal mutations or homozygous severe nondeletional α -thal mutations

3.1.1 Pre-conception carrier diagnostics/screening and premarital counseling

The first prevention strategy which has been made available in endemic countries is premarital carrier screening followed by genetic counseling, where couples at risk are provided with information for a reproductive decision making. Detecting couples at risk before marriage has been made mandatory in several Middle Eastern and Muslim countries like Iran (7), the UAE (2), Saudi Arabia (8) and Tunisia (9) (Table 3.2). Premarital testing resulted in dramatic decrease of affected births either preventing at-risk marriages through adapting partner choice, remaining childless (10) or prenatal diagnosis where it is offered. Successful examples of preconception counseling have been reported in many countries (9, 11) with Sardinia and Cyprus as the first endemic countries to develop awareness and screening to detect carriers (1, 12) with significant decline in the incidence of thalassemia major.

Early carrier detection in high schools is also a good example of prospective prevention at the preconception level (13). This has been shown to be effective in some countries such as Canada (14) and in Southern France (Marseille) (15) and Latium (Italy) (4). High school screening

resulted in a dramatic fall of beta-thalassemia major over the years in these regions. Identified carriers kept the information and remembered their status, despite the time lapse between screening at school and reproduction time, had their partner tested and inquired about prevention options (4, 14, 15). High school screening has been carried out as a national project in some Arab countries including Bahrain (16) and Egypt (17), aiming at raising awareness among the young generation. However, in Egypt, low acceptability was observed due to limited number of premarital centers and high costs. Knowing the individuals carrier status as early as school age, allows sufficient time to understand the consequences of getting an affected child in the future and to come up with a decision calmly after considerate thinking. Although school screening programs sounds feasible, they are not widely implemented. A drawback includes the risk of ethnic discrimination between the students, since the recessive diseases may be more common in people of specific ethnic origin or tribe compared to others (2).

Should premarital screening be made compulsory in Oman? And if so, should a marriage at risk be interfered by the legal system? In China, premarital genetic examination was mandated by law in 1995 and couples at risk were not permitted to marry without contraception or tubal ligation (18). This rather awkward law was redrawn as a flagrant violation of medical ethics and human rights, and premarital testing became voluntary in 2003 (19). It should be emphasized that in western countries in particular, many couples have children without a formal or legal marriage. This is unlike in Muslim communities where couples are not allowed to have children unless legally married. Although premarital screening has shown some positive outcomes in regions where the test has been mandatory, on its own is not sufficient enough to be the basis of a national prevention program if prenatal diagnosis and medical abortion are not considered as part of the process.

3.1.2 Carrier diagnostics early in pregnancy, prenatal diagnosis and pregnancy termination

In most cases of recessive inherited disorders, healthy carrier couples discover their risk only when an affected child is born. Alternatively, specific testing early in pregnancy may allow prospective prevention. At this point however, only two choices are left, accepting the risk or asking for prenatal diagnosis (PD) and medical abortion in case of an affected fetus.

As mentioned above, PD is offered to the pregnant mother mainly by testing fetal cells obtained by amniocentesis (after the sixteenth week of gestation) or a few weeks earlier in pregnancy by chorionic villi sampling (20). In most developed countries comprehensive programs for PD to address severe inherited conditions are available (21). In case of hemoglobinopathies, PD is mostly relevant in countries endemic for beta-thalassemia major and sickle cell disease. For alpha-thalassemia, as most forms compatible with postnatal life are mild (6), PD, as mentioned above, is only indicated for couples who are at risk of having a child with hydrops fetalis or severe HbH disease. In some cases, attempts to keep alive a homozygous alpha⁰-thalassemia fetus have been made by intrauterine transfusions and post-natal chronic transfusions (22). Due to the ambivalent effectiveness of such management, this practice is subject to ethical question. Examples on hemoglobinopathy (HBP) cases on which PD may be necessary is summarized in Table 1.

PD and medical abortion has shown to be the most effective prevention option available either as a national program or on request in endemic countries such as Hong Kong, Taiwan, India, Iran, Maldives and Singapore as well as non-endemic countries such as Australia, New Zealand, North America and parts of the Caribbean (23). A remarkable success in the decline of β -thalassemia major cases in regions where high thalassemia carrier rate exist were observed in northeast Thailand (24), Greece (25), Sardinia (26) and Cyprus (27).

Screening early in pregnancy is not offered routinely at the national level in Arab countries. This might be because it is socially not always accepted or due to lack of education about PD among the health workers and the population at large (1). Moreover, screening early in pregnancy leaves medical abortion as the only prevention option. This option however, is not legally or religiously permitted in many Muslim countries since, as previously mentioned, most Islamic institutions prohibit pregnancy termination at any time, unless the life of the pregnant woman is threatened. In some countries, (see below) it is allowed before the 120th day of gestation under specific circumstances (28).

Under civil law, selective abortion of an affected fetus is permissible in some Muslim countries such as Tunisia (29), Egypt (30) and Iran (31) with the last representing one of the classic examples of dramatic reduction of severe HBP incidences (32). Another recent prevention success have been reported from Northeastern Iraq (33) with 65% reduction in number of affected births over five years of implementing premarital screening and PD for HBP's. Although the Quran does not explicitly state whether termination of pregnancy is permissible in case of a severely affected fetus, PD and selective abortion remains a controversial issue in different Muslim Arab cultures (28,34,35). Some countries such as Iran and Iraq are led by a Shia scholar while others such as Egypt by a Sunni scholar. Oman is the only country led by an Abadhi scholar and all have different views towards PD in terms of legality of medical abortion. A release of a fatwa in each country based on the sub-religion (Islamic sector) is necessary as it provides guidance necessary to facilitate decision-making to those who need to base their decision upon their religious believe. Moreover, when the carrier status of the couples is found during pregnancy, it might be too late for any decision, or the time to make a decision may be too limited to understand the disease and the decision may be difficult to take under stressful circumstances (15).

Deciding to interrupt a pregnancy, even if in an early fetal stage, is a difficult and an emotional process for most couples involving their moral feelings and religious believes (2). On the other hand, knowing that the fetus will have a shorter life, devastated by a severe progressive disease that can be treated but not cured and with the high likelihood of a premature death, involves parent's responsibility as well as anxiety and fear to cope with a severely affected child. Moreover, the cost of PD can be an obstacle as well as the procedure itself, which is considered invasive and carries a risk of 1-2% of a miscarriage. The recent discovery of free fetal DNA in maternal plasma has opened a new possibility of noninvasive PD by identifying informative SNPs within a gene cluster. The method may offer an alternative to couples at risk but is only applicable in on average 50% of the cases and for HBP is thus far only done on a research basis (36).

Pre-implantation genetic diagnosis (PGD) may represent an alternative prevention option to PD. It involves testing either the oocytes before *in vitro* fertilization or the early

embryo immediately after *in vitro* fertilization, allowing pre-selection and transfer of normal pre-embryos to the woman uterus (11). PGD is permissible under Islamic law, provided that the sperms and oocytes are from the husband and wife (37). The main advantage over PD is the avoidance of terminating an affected pregnancy. However, PGD is costly, laborious, psychologically and physically burdening to the woman and requires highly experienced operators for the procedure and pregnancy rates rarely surpass 30–35% (38).

Studies of the applicability of PGD have been reported mainly from Greece (39), Sicily (40), Scotland (41) Hong Kong (42), the United Kingdom (43) and Sardinia (44). In some countries PGD is only restricted to cases of infertility.

We have interviewed Omani couples at risk and studied their attitude towards PD and medical abortion. Our results reported in chapter 13 show that if PD was legalized and approved by the Omani religious authorities, this option would be most probably accepted by the Omani couples at risk as the alternatives of choice to prevent severely affected progeny, and leading to a dramatic reduction in the number of affected births in the country.

3.1.3 Newborn screening

Newborn screening (NBS) for hemoglobinopathy (HBP) allows the identification of affected infants soon after birth, allowing secondary (morbidity) prevention, offering tailored management through prophylactic treatment and vaccination and comprehensive care prior to the development of severe clinical complications (3). National systematic newborn screening for HBP has been implemented in many countries during the last decade (45) including Arab countries such as Bahrain (46), and the UAE (47). In North America (48), the UK (49) The Netherlands (50) France and other northern European countries NBS is either national or regional (51,52,53). As a consequence of NBS one may expect that parents of affected infants would become informed and aware of their risk and would follow the available prevention methods for subsequent pregnancies and an eventual decline in the number of affected birth should be noticed. However, this was not the case in The Netherlands where after 7 years of applying newborn screening, no reduction of HBP incidence has been noticed. Possible explanations could be due to counseling directing toward treatment rather than prevention, lack of awareness in poorly informed parents (54) and potential conflict of interest on the part of clinicians and institutions (55).

In a study conducted by Al Kindi et al (56) on cord blood samples from Omani neonates, high prevalence of HBP in newborns was confirmed, emphasizing the need of implementing neonatal cord blood screening as a national strategy program in the management and prevention of HBP. Although newborn screening cannot identify and alert couples at risk when they have a non-carrier newborn child, it could be a useful secondary and primary preventative tool in Oman (57).

Donor insemination and gamete donation are not acceptable in the traditional Islamic culture and gamete donation is forbidden as it involves the use of egg and / or sperm that are not from the husband and wife, meaning that the born child belongs to one parent only and to the donor person. Adoption, although encouraged in Islam, it has many ethical and religious implications.

Ongoing screening programs and their status in some Arab countries are summarized in Table 3.2.

Table 3.2. Available HBP preventive carrier screening methods for; premarital, prenatal and neonatal screenings and their status in some Arab countries. (NA = service not available). Note: newborn screening is mainly implemented for SCD and G6PD.

Country	Premarital	Prenatal & medical abortion	Neonatal
Oman	Optional	NA	optional
UAE	Mandatory	NA	mandatory in Abu Dhabi
KSA	Mandatory	NA	optional
Bahrain	Mandatory	NA	mandatory
Qatar	Mandatory	NA	optional
Kuwait	Mandatory	NA	???
Lebanon	Mandatory	NA	optional
Jordan	Mandatory	Optional	mandatory
Palestine	Mandatory	Optional	???
Iraq	Mandatory	Optional	???
Egypt	Optional	optional	optional
Tunisia	Mandatory	optional	mandatory

3.2 The molecular spectrum of mutations and pitfalls in hemoglobinopathy diagnosis

The molecular basis of hemoglobinopathies is extremely heterogeneous. Over a thousand different mutations are reported worldwide and many of them may interact in homozygous or compound heterozygous modes, producing a wide range of disorders of varying degrees of severity (Table 3.1). The mutations are regionally specific, with each country having a characteristic spectrum of abnormal hemoglobins and thalassemia mutations. The mutation spectrum in Oman is already quite heterogeneous and the recent migration of foreign laborers is bound to expand the ethnic profile of the Omani population with different variants and defects may arise in unexpected combinations.

The usual screening of carrier couples at risk of having children with a β -thalassemia major or sickle cell disease (SCD) or sickle cell thalassemia (β /S) is achieved through basic hematology methods such as cell blood count (CBC) together with hemoglobin separation and measurement on High Performance Liquid Chromatography (HPLC) or Capillary Electrophoresis (CE). However, in some cases the interpretation by these basic methodologies can be compromised and are insufficient to give provisional results, especially in cases when multiple mutation genes are co-inherited or rare variants are involved (58).

Abnormal hematology readings and Hb separations should always be further investigated during premarital screening and risk prediction, including for the large share of non-Omani citizens residing in the country which were excluded in this project.

As mentioned in Chapter 1, the standard reading of HPLC value of 30-40% HbS will usually indicate a HbS carrier status (59). Nonetheless, some rare abnormal hemoglobins (at least 33 variants) co-migrate or co-elute like HbS with identical retention time on HPLC (60, 61).

Although the provisional diagnosis of HbS carrier made by traditional HPLC or CE is quite robust, and a second confirmatory test such as solubility test or the simple sickle test might be sufficient in most cases, in rare cases DNA analysis is essential to avoid pitfalls and to confidently report the risk assessment when more complex genotypes are present. (58).

The pitfalls: Few examples on problematic cases are for instance those of carriers of unstable hemoglobin variants either undetectable or with isoelectric point and hydrophilic interaction identical to HbA and not separable with any of the available technologies. These variants may cause severe conditions in combination with β -thalassemia or HbS (58) and will never be diagnosed unless DNA analysis is done. The differential diagnosis of hemoglobin variants in homozygous or hemizygous states (Hb variant/ β -thalassemia) is also crucial as the conditions cannot always be diagnosed by basic hematology methods. In adults, the microcytic hypochromic red cell indices and the HbA₂ measurement might help to discriminate to some extent, but not during newborn screening when HbA₂ is not yet expressed.

Another problematic cases can be those in which a β^0 -thalassemia point mutations is present in one parent while an unknown deletion in the β -globin gene cluster (normal HbA₂ and elevated or normal HbF) is present in the other. This may result in a progeny affected with mild or intermediate or a severe phenotype, depending from the type of deletion (with or without significant HbF expression). Deletions involving not only the β but also both γ -globin genes may result in severe compound heterozygosity due to the absence of the compensatory effect of fetal hemoglobin (62).

As mentioned in Chapter 1, association of β - and δ -thalassemia may also lead to misdiagnosis. That's why it is important to consider δ -thalassemia during β -thalassemia screening when borderline/normal HbA₂ value are observed risking false-negative results in the detection of couples at-risk (63).

Although the α^+ -thalassemia trait is very common in Oman and it does not represent a major burden for public health in the native population, coexisting α -thalassemia could make the diagnosis more complex (58). For instance, α^+ -thalassemia is important to predict to some extent the modulation and prognosis of the β -thalassemia and SCD patients. Conversely, α^0 thalassemia common in immigrant populations living in Oman may either improve β thalassemia major, or generate intermediate or severe phenotypes in couples of Asian origin.

Defective α -globin genes alongside the common mutations in any population generates atypical combination of these mutations with variable phenotype severity making molecular diagnostics at the DNA levels crucial especially in risk assessments. Moreover, alpha-thalassemia is not always easily diagnosed at the hematological level as it does not have specific characteristics on electrophoresis, HPLC, or CE except for a marginal reduction in HbA₂ expression (64) or in case of α -variants with known peaks (e.g.: Hb Constant Spring) and HbH disease, which may be associated with a rapidly eluting but unstable and disappearing HbH (β_4) fraction (65). On the other hand α thalassemia can be easily diagnosed if screened at birth by the presence of detected Hb Bart's (γ_4) fraction.

Finally, although most of the hemoglobin variants are either rare or silent and few semi-dominant or highly unstable, they are often not easily detectable with any of the available

basic technologies and need to be characterized at the molecular level (66). Then, in order to overcome most of these pitfalls, it is necessary to cover the full mutation spectrum in the country and keep in mind that new, rare or unexpected mutations will continue to be found in a multi-ethnic population and that consanguinity will increase the chance of homozygosity for rare conditions. Some examples of basic diagnostics and possibly associated pitfalls using the common parameters are given in Table 3.3.

Table 3.3. Summary of the basic hematological parameters used to obtain a provisional or definitive diagnosis of the most common HBP traits and factors that may influence the interpretation of the results.

Hematological reading	Diagnosis interpretation/ discrimination	External factors that may alter the hematological value
RBC	↓ = Iron deficiency, ↑ = thalassemia	Folic acid insufficiency
MCV – MCH	↓ = Iron deficiency or thalassemia	Coexisting vitamin B12 deficiency
Erythromorphology	Typical in Thalassemia	Old specimen
Osmotic fragility	Lower in Thalassemia	Old specimen
Sickle test	Positive in trait and disease	Old specimen, technical failure
Elevated (HbF)	δβ- or γδβ-thalassemia or HPFH	Raise due to HbA1c overlapping
RDW	↓ = thalassemia, ↑ = iron deficiency	Cardiac and hepatic conditions
Elevated HbA ₂	β-thalassemia, HbE, Hb Lepore	HIV patients / glycated HbS
Boarder line HbA ₂	Normal HbA ₂ β-thalassemia	δ defect or α-gene variants
Low HbS value	Coexisting α-thalassemia in HbS carrier [HbS<35-40%→(-α/αα), HbS-25-35%→(-α/-α) or (-/-αα), HbS<25%→HbH (-α/-)] or non HbS variant eluting at the same spot or iron depletion.	Transfusion with an HbS blood donor carrier in a HbA/A individual and vice versa.
Low HbC or HbE	Coexisting HbH disease	HbH instability
Hb Bart's (γ ₄) at birth	Hb Bart's of 0-5%→(-α/αα), 5-10%→(-α/-α) or (-/-αα), 10-30%→HbH (-α/-).	Old sample

3.3 The process of changing attitude

As mentioned, the attitudes of the Arab-Muslim countries toward genetic screening, prenatal diagnosis (PD) and medical abortion is changing in some areas as more people are educated and aware of the consequences. In other countries screening and primary prevention remains limited due to many factors such as low economic resources, limited premarital screening centers, religious beliefs, culture and traditions, literacy, education and government policy.

If no preventative measures are taken in these endemic regions, in time the cost of treatment would consume the entire health budget of the country. However, before establishing any prevention option, it is necessary to assess the interest of the community in a preventive service and to generate professional genetic counselors that understand the sensitivity and the

ethical issues in handling prevention options such as PD and pregnancy termination and finally is essential to have policy makers aware of the magnitude of the problem.

Efforts are then needed to establish a comprehensive infrastructure for pre-conception clinics as an essential community based primary prevention measures (11) and to stimulate general practitioner and midwives to change their passive attitude of “no complaints = no actions” into an active behavior of referral for carrier diagnostics for prevention purposes. Finally, to avoid stigmatization, awareness campaigns should be directed to the whole of the population rather than to immigrants or tribes/ethnic groups at high carrier frequency.

A number of studies have examined the attitude of Muslim couples at risk towards early genetic screening, PD, medical abortion and PGD and variable results were obtained. A recent study conducted in Saudi Arabia, showed that 90% of the couples at risk who were diagnosed prior marriage and advised to choose a different partner, proceeded with their marriage intention (8). When these couples were questioned, around 50% thought it is a good thing to undergo a screening test but before the engagement stage because of the difficulties in cancelling familial commitment, the wedding ceremony preparation, and because of social stigma (8). Similar cultural feelings have been observed in neighboring Middle Eastern countries, as well as the desire to have early preventive genetic services to reduce the incidence of HBP in these regions with high frequency of consanguineous marriages. Studies from Saudi Arabia (67) and Lebanon (68) have shown that couples considered PGD to be preferable, as their opinion towards PD was influenced by the religious authorities in the country.

Not all Muslim cultures accept the Fatwa of the official Islamic jurisprudence allowing selective abortion within the first trimester of pregnancy in case of an affected foetus (Council of the World Islamic League, 15-22/07/1410 Hijri/ 10-17 February 1990). However, all Muslim jurists, have agreed regardless the different Islamic streams that PGD for genetic disorders is permissible provided that the gametes are from husband and wife and this on the basis that IVF does not conflict with God’s desire nor is considered a modification of God’s creation, but rather a way of treatment because PGD and embryo selection is done when embryos are only at the eight-cell stage (69). The question is why should PD, followed by early medical abortion in vivo within 120 days of conception be unacceptable to all Islamic sectors, while PGD which could be considered in fact an early medical abortion in vitro be acceptable? In both cases there is interruption to fetal growth to prevent the birth of a severely affected child.

3.4 Diagnostics and management of hemoglobinopathies in Oman

Since sickle cell disease (SCD), β - and α - thalassemia are the most common autosomal recessive gene disorders in Oman, premarital testing for hemoglobinopathies (HBP) is provided as a national program for identifying couples at risk. The service is voluntary and is offered free of charge to the Omani citizens. The first line of diagnostic tool is based on the measurement of the hematological parameters (CBC) and on the separation and estimation of the hemoglobin fractions on high performance liquid chromatography (HPLC).

A normal individual after the age of 2 will present with about 96-97% HbA, $\pm 3\%$ HbA₂ and $<1\%$ HbF. Any change in this pattern will be anomalous and might indicate an HBP disorder. Frequent traits are confirmed by simple additional analysis, solubility test for HbS, alkaline or acid electrophoresis for common traits (HbD, HbE, HbC). If the variant peak cannot be

confirmed biochemically, DNA test is requested. Figure 3.1 summarizes how the value of HbA₂ is interpreted in the Omani population.

Molecular analysis is used to confirm a diagnosis when hematological and biochemical parameters are complex or unclear. The main technologies involved are direct DNA sequencing for the β - and α - globin genes. Gap-PCR is used to detect the common α -thal deletions when microcytic and hypochromic red cell indices are observed. Multiple ligation probe amplification (MLPA) is the ultimate solution for defining unknown deletion defects of either the β - or α -globin genes cluster and should be considered to be implemented in the genetic laboratory.

For the development and quality of a molecular laboratory one needs to keep up with new technologies and scientific advances. Robust and fast screening methods should be used once premarital testing for HBP becomes mandatory in the country. The referral hematology laboratory should have more than one method for hemoglobin analysis besides HPLC, for example Capillary Electrophoresis (CE). The serum ferritin test should be replaced by the zinc protoporphyrin method and be made mandatory on all samples. Serum ferritin is usually normal or elevated in β -thalassemia carriers who have increased iron absorption in the intestine but might also be borderline or low in α -thalassemia carriers. Moreover, ferritin is an acute-phase protein and might be falsely elevated in the presence of a coexisting infection (58). In some clinics, genetic counseling is not provided by a certified professional genetics counselor, but by a local health practitioner who has sufficient experience and training to convey the results. Efforts should be directed to have more professional specialists in the field.

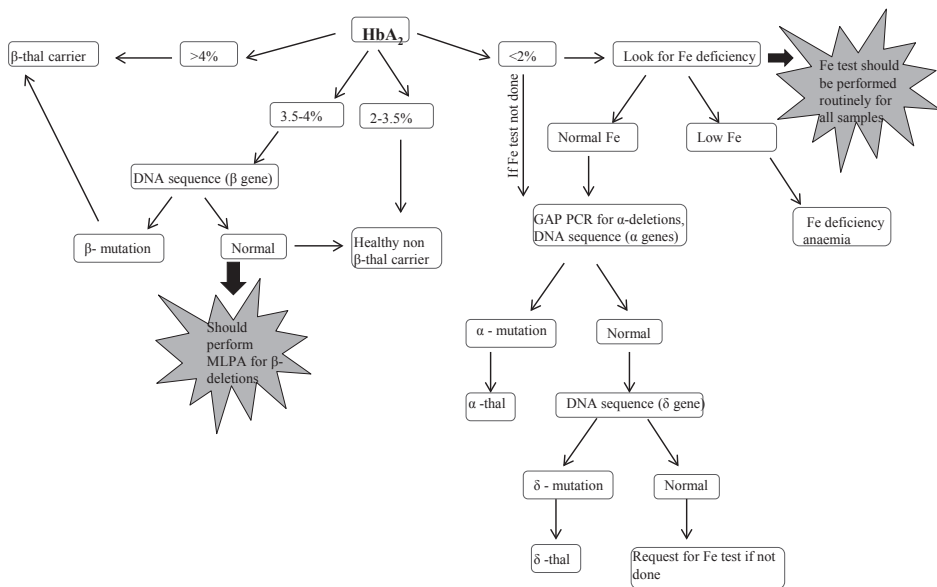


Figure 3.1. The criteria used to diagnose HBP related cases based on HbA₂ value, indicating when DNA analysis is performed. Tests highlighted in gray are needed and should be considered for future implementation in Oman.

Currently prevention of SCD and β -thalassemia major (BTM) in Oman involves either choosing another partner, accepting the risk or seeking for prenatal diagnosis (PD) or PGD abroad for those who can afford the costs. A typical premarital screening scenario includes the following stages: signing a consent form at the first consultation, sending a blood sample to the nearest hematology laboratory, sending a sample for DNA analysis if diagnosis cannot be confirmed by hematology tests, receiving results (by fax) and finally reporting the findings to the couples by either arranging a counseling session or conveying results over the phone.

Severely affected patients are treated in the major hospitals. Thalassemia patients are offered monthly blood transfusion and chelation therapy. Sicklers are regularly immunized and admitted when severe symptoms strike. Recently, Hydroxyurea treatment has been introduced but is given to only very severe cases of SCD following stringent criteria.

The majority of the society of modern Oman may eventually accept selective abortion if religiously permitted. Prevention will spare a lot of human suffering to children and families as well as the huge expenses to the country necessary for treatment of the ever growing number of patients affected with these incurable diseases. Efforts were made to open a constructive dialogue with the main religious authority in Oman (country's Muftee) in order to discuss the issue of PD and medical abortion in case of a severely HBP affected fetus. However, to date, the religious authority has not agreed to make this service legal as yet.

A SUMMARY OF THE PATIENTS AND METHODS

As mentioned at the beginning in "Aim of this thesis" the intention of this study was to investigate all possible factors involved with the implementation of a national prevention strategy for Oman and in other Muslim countries also endemic for HBP. For this I have studied at the biochemical, molecular and clinical level a cohort of 722 Omani subjects (1444 alleles), affected with BTM, SCD, alpha thalassemia or presumed to be carriers of HBP. Details of all patients and methods used for analysis are included in the publications summarized in Chapters 4-13.

I have provided the widest spectrum of common and rare mutations in Oman and the prevalence of specific mutations more common in specific areas of the country (Chapters 4, 5, 6, 7 and 8),

I have studied the factors involved in the modulation of severity of SCD such as haplotype and coexisting alpha thalassemia and the value of these factors for prognostics, risk assessment, state of the art treatment (genotype / phenotype correlation) and for pharmacogenomics (Hydroxyurea treatment) (Chapters 9, 10 and 11).

I have tested the application of next generation technology in identifying large number of at risk cases in a rapid period of time (Chapter 12)

Finally I have studied the attitudes of the Omani couples towards PD and medical abortion (Chapter 13).

I believe that this study has provided new information on different aspect essential for the implementation of a modern, national diagnostics, treatment and prevention program for HBP in Oman.

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