Toward prevention of Hemoglobinopathies in Oman

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CHAPTER 14

SUMMARY, DISCUSSION AND CONCLUSION

Despite well-organized pre-marital clinics and the dramatic improvement in public health in the last few decades, the birth of children severely affected with sickle cell disease (SCD) or beta thalassemia major (BTM) is still high in Oman. Patients with severe hemoglobinopathoes (HBP) and their families are facing heavy suffering while treatment requires intensive, burdening and expensive efforts to be provided by public health which is offered free of charge to Omani citizens.

For further improvement of HBP management, effective prevention programs are needed. Better detection and counseling of couples at risk should be offered to make an informed reproductive choice possible for healthy carrier couples at risk of having children with severe hemoglobin disorders. Introducing these options is complex but essential and is currently limited by the restrictions imposed upon prenatal diagnosis (PD) and medical abortion in Oman.

Developing an efficient prevention strategy for HBP is a complex process both at the cultural and at the technical level. The essential technical and cultural elements involved in the process have been studied in this thesis providing the necessary knowledge to be used for the implementation of the most suitable strategy. Fundamental in the process towards establishing the necessary technical tools is an adequate knowledge of the molecular spectrum of HBP mutations in the country.

MOLECULAR SPECTRUM

We have studied the molecular spectrum of beta- and alpha- thalassemia in a large cohort of Omani patients demonstrating the widest spectrum of mutations reported thus far in the country, including the characterization of the most common mutations in the different regions of the country (Chapters 4-7). Our analysis has shown not only a broad range of common mutations, some of which are tribe specific and indicating a founder effect and genetic drift, but also many less common or rare mutations associated with historical migration patterns. From these studies, at least 32 different β-thal determinants were found among the Omanies (Table 5.1) with one novel beta-globin gene transversion in the promoter region (Table 6.2). In regard to the alpha globin gene, at least 21 defects were found with two novel mutations in the alpha 3.7 hybrid gene (Table 7.5). In Chapter 8, a total of 10 delta gene defect was characterised in the studied cases among which 2 novel delta determinants were found (Table 8.1). The necessity to characterize the molecular spectrum of delta globin gene defects is important in countries with high rate of beta thalassemia, especially when diagnosis of β-thalassemia carrier is based upon the measurement of HbA_2 fraction only as this can be overlooked when it is co-inherited with a delta mutation.

The broad range of HBP defects identified in these studies (Chapters 4-8), demonstrate the existence of a heterogenic pattern in the country accounting to gene-flow and the past trade with other countries. Molecular detection of hemoglobinopathies is essential for providing accurate genetic counseling following premarital screening as well as in tailoring treatment plan in affected patients.

Moreover thorough characterization of the spectrum of common and rare beta- and alpha- globin gene mutations in the Omani ethnic group allows the selection and application

of the most appropriate current molecular technologies. In addition the molecular spectrum is essential for genotype/ phenotype prediction and risk assessment, for treatment and prenatal diagnosis and also for designing advanced molecular methods for future application to detect all mutations simultaneously. For the latter we evaluated the use of the Ion Torrent PGM in beta thalassemia diagnostics (Chapter 12). Based on our findings, we have concluded that Ion Torrent could be a convenient sequencing platform for large scale diagnostic screening in Oman during premarital or in early pregnancy testing.

PHENOTYPE PREDICTION

Predicting the severity of the disease is not always straightforward. Also when the only available option is adapting partner choice, an accurate knowledge of the molecular spectrum examined in the totality of the genotype, is of great importance for a thorough premarital counseling of couples at risk. Therefore we have focused upon a number of technical elements essential for risk prediction and counseling and we have been able to show the following. Some defects, mild or silent, that cannot be easily detected at the hematological level may generate complex genotypes in association with the beta and/or alpha globin genes (Chapter 6).

Delta gene defects occur at a considerable frequency in Oman and may interfere with the diagnosis of beta thalassemia using hematological parameters (Chapter 8).

The co-existence of alpha thalassemia, very frequent in Oman, may modify the phenotype of the progeny of couples at risk and make risk prediction more complex. Therefore alpha thalassemia should not be overlooked during premarital screening when diagnosis is made on hematological parameters alone. If the couples are carriers of an alpha° or a relevant alpha+ defect, there is a 25% risk of getting a hydrops fetalis or severe HbH disease in the child and it is therefore necessary to perform molecular tests to define the alpha thalassemia defect and to differentiate between iron deficiency and/or alpha- or delta- thalassemia (Chapters 6 and 7).

PHENOTYPE PREDICTION AND CURE VERSUS TREATMENT

Treating a disease does not always lead to a cure. This is in general the case for most genetic disorders and for HBP's in particular.

Different HBP phenotypes may require different treatment and the only "cure option" for severe HBP is a successful stem cell transplantation using a HLA identical sibling as the donor. This expensive option can reduce morbidity and prolong survival but is not easily accessible to the great majority of HBP patients in Oman. Therefore we have focused in this thesis upon elements involved in modulating phenotype and influencing the efficacy of treatment.

We have conducted a countrywide analytical study on the correlation between the genetic makeup (beta-cluster haplotype, coexisting alpha-thalassemia and XmnI polymorphism) and phenotype (Chapter 9, 10 and 11) in patients with sickle cell disease (SCD).

Given the genetic heterogeneity of the Omani population, many different beta-cluster haplotypes were identified in SCD patients, and therefore we have explored the genotype-phenotype correlations which is important for healthcare providers so that better disease management of patients can be developed (Chapter 9).

We have further observed that the hyroxyurea drug (HU) can ameliorate the severe phenotype of the large majority of Omani SCD patients and that the XmnI C>T polymorphism is highly associated with a positive response to HU treatment. Few patients that did not show improvement after treatment with HU even after increasing the dose were all carries of an identical compound heterozygous beta gene cluster haplotype (Chapter 10). Thus we conclude that the presence of Xmn I polymorphism in Omani SCD population is a predictor of response to HU but other factors such as either haplotype and/or sub-haplotype are also involved. These findings allow an early planning for an alternative treatment for those who are less likely to respond to the drug.

Moreover we compared the clinical severity of SCD patients of identical homozygous HbS/S genotype that are categorized by identical beta globin cluster haplotype with different alpha globin genotypes and shown that alpha-thalassemia can modulate the hematological picture of Omani SCD patients but not clearly the overall severity manifestation in patients of all haplotypes (Chapter 11).

Overall, our results suggest that multiple determinants are involved in predicting disease severity in SCD patients and that these determinants should be considered for better assessment and treatment. Drawing correlation lines between the genetic make up and clinical severity is essential for prognostic purposes, accurate diagnosis and thus planning for the best tailored treatment.

PREVENTION OPTIONS

Primary prevention has been achieved in different countries through information and screening at different levels. School, premarital, early pregnancy and neonatal screenings have been applied with different results. The approach involving screening early in pregnancy followed by PD and eventually selective medical abortion has shown to be the most effective and the most accepted prevention method, lowering the incidence of severe HBP cases in many countries. Therefore in this thesis we have studied the attitude of a representative Omani cohort toward prevention by PD and medical abortion in view of the ethnic, cultural and religious backgrounds (chapter 13).

In our survey we have found that the majority of the couples inquired would have chosen for PD if this service would have been available in Oman but only if termination of pregnancy would be approved by law and by the country's main religious Mufti.

The issue of PD and selective termination of pregnancy in case of an affected fetus has been debated and legally approved in different Muslim countries but for the time being, early population screenings and the advice not to marry for carrier-couples at risk are the only available preventative measures in Oman. However, often the advice not to marry is ignored and high rates of both consanguinity and HBP frequency would benefit from the acceptability and availability of PD and medical abortion.

The results of our enquiry and the incidence of the diseases in the country show that these issues should be discussed and solved with the approval of the country's Islamic leader (Mufti) and public health authorities and that appropriate prevention measures should be offered to the many couples at risk in the country. Moreover, public health authorities should be

concerned with the awareness of the population and should educate couples at risk on the availability of alternative preventative options.

IN CONCLUSION

This thesis has investigated most of the technical aspects necessary for a state of the art prevention programme for HBP in Oman. Currently the most rational way to offer appropriate prevention in Oman, whilst simultaneously respecting cultural and religious aspects, is to provide the relevant information and screening at the preconception level because it leaves either the option not to marry or to seek PD and medical abortion abroad (the latter for those couples who can afford it). With appropriate infrastructure this strategy could dramatically reduce the incidence of severe hemoglobinopathies in Oman and herewith, the suffering of patients and parents and the need for long lasting supporting therapy with the associated financial burden on the public health budget.