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REVIEW ARTICLE

Haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in a Scandinavian perspective

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Abstract
Haemoglobinopathies (mainly thalassaemia and sickle-cell anaemia syndromes) and glucose-6-phosphate dehydrogenase deficiency (G6PD) are globally among the most prevalent single-genomic diseases. About 3 % of the world's population are heterozygotic for β-thalassaemia and about 1–2 % for sickle-cell anaemia, and it is estimated that more than 400 million people are affected by G6PD deficiency worldwide. The disorders are most prevalent in the Mediterranean area, in Asia and Africa. The Scandinavian countries, among others, have seen a boom in immigration during the past 20 years, and therefore migration makes haemoglobinopathies as well as G6PD deficiency increasingly more important from a differential diagnostic perspective in most countries. The purpose of the present special issue of the Journal is to summarize current epidemiological data and elucidate trends and practices in the laboratory diagnosis of these disorders.

Key Words: *G6PD, glucose-6-phosphate dehydrogenase deficiency, haemoglobin, haemoglobinopathies, thalassaemia*

Introduction

Haemoglobin is among the molecules in nature best tailored for the purpose of transporting oxygen to and carbon dioxide from the tissues, and buffering changes in blood proton concentrations. Easily accessible to scientists in high concentrations, highly biologically active, but not overly structurally complex, it is probably the most thoroughly studied protein known. Adult haemoglobin A (HbA) is a tetramer of two α and two β globin chains each containing an iron-binding haem moiety. Normally, the equal rate of synthesis of the two α and two β globin chains is tightly coordinated (balanced) within a range of only 0.1 %.

Haemoglobinopathies constitute a family of diseases sharing the common pathophysiological cause of defects in the genetic blueprint of the globin parts of haemoglobin. According to pathophysiological mechanisms, haemoglobinopathies are of two types: 1)

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those caused by inherited structural alteration in one of the globin chains leading primarily to abnormal physical properties of the haemoglobin, e.g. sickle-cell anaemia, and 2) inherited defects in the rate of synthesis of one or more of the haemoglobin chains leading to ineffective erythropoiesis, haemolysis and variable degree of anaemia (thalassaemia).

Historical developments

Already in 1910 Herrick pioneered in observing sickled cells in the blood of an anaemic African graduate student [1]. Subsequently, it was shown that the erythrocytes sickled when exposed to low oxygen tension [2,3]. In 1949 and the years thereafter, the genetic and molecular background of the haemoglobinopathies was elucidated when it was shown that haemoglobin-S is the abnormal protein causing sickle-cell anaemia [4–7]. This was in fact the first comprehensive demonstration of a molecular disease in the pure sense of the concept.

In 1925, Cooley & Lee [8] reported the childhood anaemia, splenomegaly and bone changes associated with thalassaemia. The name ''thalass anaemia'' (later thalassaemia) was suggested in a more comprehensive report of the pathologic findings of Whipple & Bradford [9].

Subsequently, a large number of mutations in the haemoglobin molecule have been discovered. Most of the mutations do not lead to disease, but some, especially the thalassaemia and sickle-cell anaemia syndromes, are very prevalent, making haemoglobinopathies among the most common hereditary diseases on earth [10].

The genetic mutation of thalassaemias leads to 50 % reduction in the risk of malaria, whereas sickle cell mutations lead to 90 % reduction in the risk [11]. Historically, this is likely to have increased the prevalence of haemoglobinopathies in populations living in geographical locations infested by malaria parasites.

G6PD deficiency was discovered in the 1950s, and was shown to be the cause of the haemolytic effect of primaquine [12]. Today, we know that precipitating factors such as infections and consumption of fava beans can elicit severe haemolysis in patients with G6PD deficiency. In addition, we know that G6PD deficiency is an major cause of neonatal jaundice. Geographic co-localization studies and population studies indicate that G6PD-deficient alleles confer some protection against severe malaria caused by P. falciparum, probably in the order of 50 % risk reduction [13].

The thalassaemias

Thalassaemias are due to deficient production of α- or β-globulin. The defects are autosomal recessively transmitted, which means that the heterozygous individuals are near asymptomatic carriers (thalassaemia minor) while the homozygous individuals have varying degrees of symptoms, ranging from very few symptoms via thalassaemia intermedia to the full-blown thalassaemia major. The most severe cases of β-thalassaemia are characterized by the accumulation of excess α-globin chains in the erythroid precursors. This leads to substantial increase in erythroid apoptosis which in turn causes ineffective erythropoiesis. The β-globin chains accumulating in the erythrocytes in the most severe cases of α-thalassaemia cause much less apoptosis and ineffective erythropoiesis. However, the accumulated β-globin chains are susceptible to oxidative denaturation, which damages the RBC membrane and leads to haemolytic anaemia (Haemoglobin H disease).

Improved understanding of the pathophysiology of thalassaemias has already resulted in improved therapeutic options, including: transfusion programmes, better control of the iron overload caused by repeated blood transfusions and increased iron absorption, and the option of bone-marrow transplantation. Transfusions of erythrocytes are needed to avoid death from cardiac failure in the most severe cases. However, each unit of erythrocytes contains 200–250 mg of iron, and the body has only passive mechanisms removing stored iron which are not influenced by the total body stores of iron. Iron accumulation induces the formation of free oxygen radicals leading to cell and organ damage, primarily involving the heart, liver and the endocrine system. Regular transfusions of patients therefore require therapy of iron chelating in order to remove the iron. Unfortunately, the choices of chelators are limited and the treatment is troublesome and not free of complications and compliance problems [14,15].

If an alternative gene in the β-globin cluster, e.g. the gamma globin gene, could be turned on, it would combine with the excess of alpha globin chains and prevent its accumulation. Therefore an important therapeutic endeavour concerning the β-thalassaemias involves efforts to turn on these gamma globin genes.

Sickle cell diseases

Sickle cell haemoglobin is a mutated form of haemoglobin where the amino acid valine has been substituted for the glutamic acid normally present as the sixth amino acid in the β-globin chain. This seemingly minute change has the dramatic effect that the haemoglobin polymerizes and becomes poorly soluble when the oxygen tension in the blood is lowered. The homozygous sickle cell anaemia is the most severe form of the sickle cell diseases. Less severe forms are associated with the heterozygous form combined with β-thalassaemia, haemoglobin C or in rare cases more uncommon mutations in the β-globin chain.

Glucose-phosphate dehydrogenase deficiency

G6PD catalyses the first step in the pentose phosphate pathway involving the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone leading to the reduction of $NADP^+$ to NADPH. This compound is of crucial importance in the protection of red cells from oxidative damage. In G6PD-deficient red cells, oxidative stress may lead to haemolysis, since these cells are unable to ensure the formation of NADPH at a sufficient rate. G6PD deficiency is linked to the X-chromosome and therefore the large majority of affected individuals are males, although there are affected females where the prevalence is high.

Prevalence of haemoglobinopathies and G6PD deficiency in the world

Despite the more than 500 known variants of haemoglobin, the two most important in terms of prevalence, morbidity and mortality are β-thalassaemias and sickle cell anaemia. It has been estimated that up to 7 % of the world's population are carriers of an inherited haemoglobin disorder [16]. The thalassaemia syndromes are mainly present in the thalassaemic belt, e.g. on both sides of the Mediterranean, in the Middle East, Pakistan, India and in Southeast Asia, including the Philippines and Indonesia. More than 80 % of

all patients suffering from sickle cell anaemia live in Central Africa, but it is also prevalent in the Arabian Peninsula, in the Middle East, the Indian subcontinent and the eastern part of the Mediterranean.

G6PD deficiency is the most frequent hereditary enzyme deficiency in the world. It is estimated that more than 400 million people are affected worldwide. Its world distribution is similar to that of the thalassaemia syndromes and remarkably similar to the world distribution of malaria, except for southern Europe. However, malaria in southern Europe has only been eradicated in recent times.

A substantial proportion of the recent immigrants to Scandinavia come from the areas where thalassaemia and sickle cell anaemia are prevalent (Figure 1).

Migration and the prevalence of haemoglobinopathies in the Scandinavian countries

The Scandinavian countries had substantial net emigration from the mid-nineteenth century to after the First World War. The flow was reversed after the Second World War by

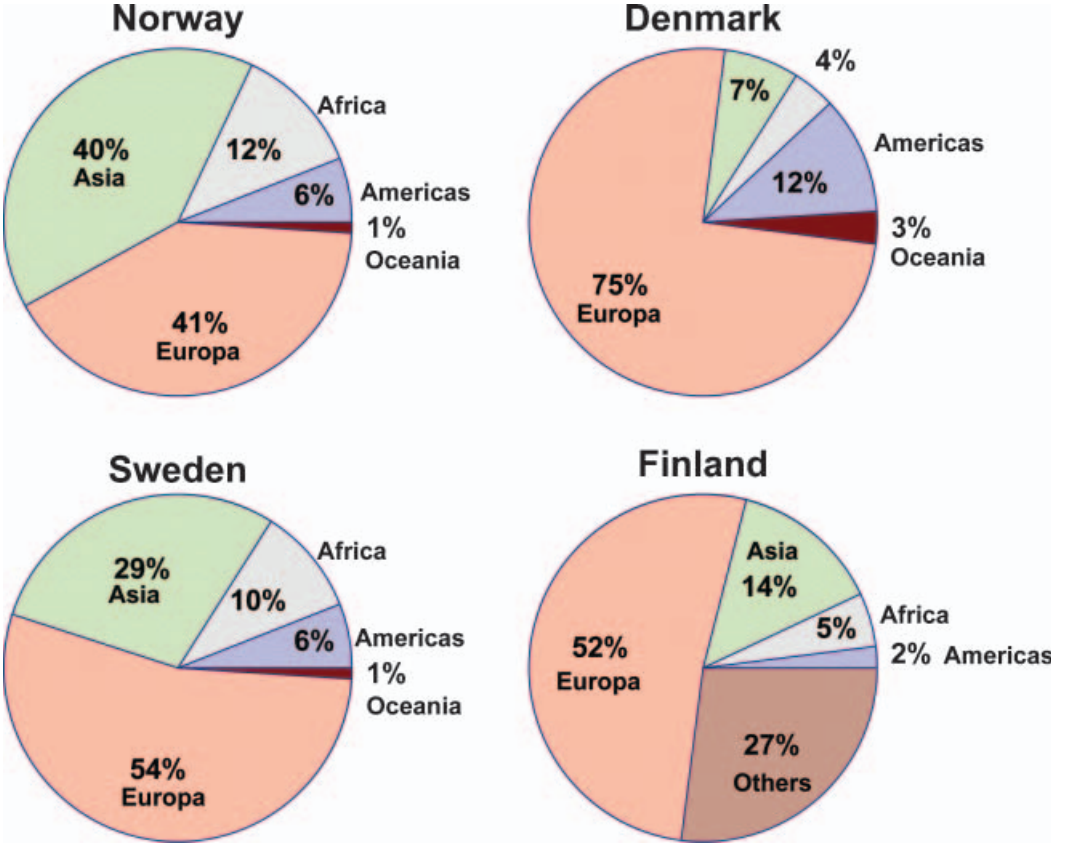

Figure 1. Regions of origin of immigrants to the Nordic countries during the recent decade. Statistics for Norway from http://www.ssb.no/, Denmark from http://www.dst.dk/, Sweden from http://www.scb.se/ and Finland from http://www.stat.fi/. In the period 1995–1999, the net annual immigration to Norway was 18,000, Denmark 16,000, Sweden 53,000 and Finland 10,000.

net immigration, at first from European countries but increasingly from other countries of the world [17–20], in particular from countries with a high prevalence of haemoglobinopathies. Considering the substantial increase in the number of immigrants from regions of the world with a high prevalence of haemoglobinopathies, the number of cases treated in Scandinavian hospitals is still comparatively low (Figure 1 and Table I). One reason for this may be that healthy people are more likely to emigrate compared to their sick counterparts.

It has been estimated that about 2–4 % of the immigrant population in Copenhagen County is heterozygous for either β-thalassaemia or sickle cell anaemia [21,22]. In

Table I. The ICD-10 (http://www.who.int/classifications/icd/en/) diagnosis of haemoglobinopathies and related diseases in patients hospitalized in Sweden (population of 9 million) in the period 1998–2003. Statistics kindly provided by Annika Edberg at the Swedish National Board of Health and Welfare.

Diagnosis	1998	1999	2000	2001	2002	2003	1998–2003
D55.0 Anaemia due to glucose-6-phosphate dehydrogenase [G6PD] deficiency	4	10	7	4	5	8	**33**
D55.1 Anaemia due to other disorders of glutathione metabolism			2				**2**
D55.2 Anaemia due to disorders of glycolytic enzymes	1	2		2			**5**
D55.9 Anaemia due to enzyme disorder, unspecified	6	5	4	5	5	6	**31**
D56.0 Alpha thalassaemia	2	1	3	2	1	4	**12**
D56.1 Beta thalassaemia	17	18	12	15	19	17	**46**
D56.2 Delta-beta thalassaemia			0	1			**1**
D56.4 Hereditary persistence of fetal haemoglobin [HPFH]		1			1		**2**
D56.8 Other thalassaemias		2	1	1	1	1	**6**
D56.9 Thalassaemia, unspecified	16	9	10	8	9	14	**53**
D57.0 Sickle-cell anaemia with crisis	9	12	17	12	14	17	**38**
D57.1 Sickle-cell anaemia without crisis	13	8	19	11	14	12	**45**
D57.2 Double heterozygous sickling disorders	1	2	1	1	1		**6**
D57.3 Sickle-cell trait	2		1		1		**4**
D57.8 Other sickle-cell disorders	1		1	1		1	**3**
D58.0 Hereditary spherocytosis	31	34	34	30	40	32	**155**
D58.1 Hereditary elliptocytosis	2	1					**3**
D58.2 Other haemoglobinopathies	2	4	2	2	1	1	**10**
D58.8 Other specified hereditary haemolytic anaemias	2	1			2	1	**5**
D58.9 Hereditary haemolytic anaemia, unspecified	7	6	3	4	7	11	**33**
D59.0 Drug-induced autoimmune haemolytic anaemia	10	16	7	6	6	7	**49**
D59.1 Other autoimmune haemolytic anaemias	100	111	99	110	103	132	**523**
D59.2 Drug-induced non-autoimmune haemolytic anaemia	4	6	8	2	5	4	**29**
D59.3 Haemolytic-uraemic syndrome	21	14	19	15	26	28	**108**
D59.4 Other non-autoimmune haemolytic anaemias	18	24	19	12	17	15	**88**
D59.5 Paroxysmal nocturnal haemoglobinuria [Marchiafava-Micheli]	5	3	2	1	4	5	**15**
D59.6 Haemoglobinuria due to haemolysis from other external causes						2	**2**
D59.8 Other acquired haemolytic anaemias	7	2	3	2	3	2	**19**
D599 Acquired haemolytic anaemia, unspecified	132	103	105	111	93	101	**591**
D55–D59	388	366	354	334	359	391	**1730**

Denmark, the National Board of Health recommends screening of pregnant immigrants with origin in areas where haemoglobinopathies are endemic (Sundhedsstyrelsen. Svangreomsorg, Retningeslinjer og redegørelse, Copenhangen 1998).

The incidence of ICD diagnoses of haemoglobinopathies in hospitalized patients in Sweden in the period 1998–2003 is given in Table I. Overall, the incidence is stable during this time period.

In Norway, there are no official data on the prevalence of haemoglobinopathies nor an annual registration of new cases within each diagnostic group. A small study from 2001 [22], however, does show that in 1997 48 patients were given a haemoglobinopathy diagnosis, mainly β-thalassaemia minor ($n=32$) and β-thalassaemia major ($n=8$). This is certainly an underestimation, since the finding was based on the response to a questionnaire sent to 149 departments of paediatrics, gynaecology and internal medicine, with only 80 % response. The study does indicate, however, that the number of new cases was low in 1997.

In a more recent survey, we found that 278 new cases of haemoglobinopathy were diagnosed in Norway in 2005, as indicated in Table II. The data originate from each of the five laboratories performing biochemical diagnoses of haemoglobinopathies (haemoglobin-typing, gene-testing of α-thalassaemia, sequencing of the β-globin gene) in Norway and should therefore give a reliable picture of the situation in 2005. A few other laboratories specializing in gene-testing also test for α-thalassaemia, and this activity is not included in the data given in Table II. The number α-thalassaemias may thus be slightly under-estimated.

The increase in cases from 1997 to 2005 is certainly to a large extent due to an increased medical focus on the diagnosis of haemoglobinopathies in the immigrant population during the past few years. In one of the five laboratories the number of requests for biochemical testing for haemoglobinopathies increased by 225 % in a 5-year period. The number of immigrants in Norway has increased in recent years, but not to the extent that this alone can explain the enhanced diagnostic activity.

This study also shows that the diagnosis was verified in approximately 30 % (in 248 of 749 patient samples) of all patients investigated for a possible haemoglobinopathy.

In Norway, with its total population of 4.5 million, there are approximately 240 000 immigrants from regions with a high prevalence of hemoglobinopathies. More than 50 % of these immigrants live in the Oslo region (18 % of the population in Oslo). Awareness of this important health problem in the immigrant population has increased in recent years,

Table II. Number of diagnosed cases for haemoglobinopathies in Norway in 2005. Since some laboratories use methods that do not separate between HbE and HbD, these are presented as a group. The various α-gene deletions are not specified.

Diagnosis	No. of cases/2005
β-thalassaemia minor	148
β-thalassaemia major	0
HbS heterozygote	17
HbS homozygote	15
HbC heterozygote	3
HbD or E heterozygote	5
HbD or E homozygote	6
α-thalassaemia	79

especially in the Oslo region. Haemoglobinopathies as a diagnostic challenge will certainly attract more attention and resources in forthcoming years.

Conclusions

Globalization, while increasing competition-related adjustments, is a major factor fostering understanding between individuals and nations around the world – among other things through its effects on migration. Globalization and migration not only change service and industry but also the local prevalence of both inherited and acquired diseases. We are becoming increasingly aware that diseases earlier mainly considered to be a local problem, from a diagnostic and therapeutic perspective, are now global challenges. Increased clinical awareness and improved diagnostic facilities are needed to deal with haemoglobinopathies. However, advances in screening policy, prenatal diagnosis and the development of new pharmacological therapies and molecular biological techniques promise to dramatically reduce the burden of haemoglobinopathies on the world population if we are successful in applying them in a prudent and responsible manner.

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