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REVIEW ARTICLE: THALASSEMIA IN ASIA 2021

Thalassemia in Asia 2021 Thalassemia in Brunei Darussalam

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ABSTRACT

Acknowledging and understanding the extent of thalassemia and hemoglobinopathy issues in a country is crucial for the benefit of implementing a national preventive and control program to reduce its prevalence. In order to obtain reliable prevalence data, the gene frequencies of the thalassemias and other hemoglobinopathies should be investigated. Molecular studies on thalassemia have yet to be done for Brunei's population. It was estimated that carriers of thalassemia or hemoglobinopathies in Brunei is approximately 5.0% or less of the overall population. There are about 200 current cases of thalassemia and other hemoglobinopathies including adults and children reported across all four districts of Brunei. Blood parameter analysis, microscopy, hemoglobin (Hb) electrophoresis and high performance liquid chromatography (HPLC) are the most common methods of investigation in aiding diagnosis in the hospital laboratory. Genotyping analysis conducted in an overseas laboratory has been employed to confirm some diagnosis. Compiled data from 2009-2017 at the Hematology Laboratory of the Raja Isteri Pengiran Anak Saleha Hospital, Jalan Putera Al-Muhtadee Billah, Bandar Seri Begawan, Brunei Darussalam, showed that the most reported diagnoses are α-thalassemia (α-thal) trait, β-thalassemia (β-thal) trait, heterozygous Hb E (*HBB*: c.79G>A)/β-thal, β-thal major (β-TM) and β-thal intermedia (β-TI). The data reported indicate the importance of establishing a thalassemia registry with relevant data on patients and patient outcomes as a tool for monitoring and improving patient care.

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Introduction

Hemoglobinopathies are a diverse group of inherited blood disorders characterized by decreased expression of the functional hemoglobin (Hb) proteins. The disorder results from hereditary mutations that cause qualitative defects leading to structurally abnormal Hb variants such as Hb S (*HBB*: c.20A>T), Hb E (*HBB*: c.79G>A) and Hb Constant Spring (Hb CS or *HBA2*: 427 T>C) or quantitative defects of the globin expression as in thalassemia disorders. Thalassemia is the most common type of hemoglobinopathy. Additionally, there are also cases where Hb variants can be associated with thalassemia genotypes and may cause clinical complications [1]. The thalassemia types vary depending on which polypeptide chains' expressions are affected. The globin chains are coded by the α gene clusters (*HBA1/HBA2*) on chromosome 16 and the β-globin gene cluster (*HBB*) on chromosome 11 [2]. Decreased globin chain expressions can be caused by deletion of structural genes, or mutations that cause decreased RNA or protein synthesis or stability [3]. Where there is an imbalance of α and β chains, there is a formation of unstable tetramers and an accumulation and precipitation of unpaired chains that is followed by hemolysis, and ineffective erythropoiesis [4]. Most thalassemia cases are inherited in an autosomal recessive manner, and thus, family history is effectively part of the primary line of establishing a diagnosis.

Epidemiology

Hemoglobinopathies and thalassemias are the most common Mendelian disorder in the world. They used to be epidemiologically indigenous to specific geographical areas that are malaria-endemic regions such as the Mediterranean, African, Middle Eastern, European, South Asian and Southeast Asian regions due to natural selection [5]. However, with continual population migration, it is now distributed worldwide [6]. Some thalassemia mutations and the Hb variants are more prominently specific to their regions where each local population has its own spectrum of mutations, while some other mutations are mutual among populations [7]. Additionally, populations where consanguineous marriages are common have shown high frequency of thalassemias and hemoglobinopathies due to lack of genetic drift [8]. It has been reported

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that estimation of carriers of thalassemia genes is about 1.0–5.0% of the population worldwide [9].

Clinical manifestations

Thalassemia has been well-researched and documented in neighboring countries such as Malaysia, Thailand, Philippines, Singapore, Indonesia and Vietnam. The combinations of various Hb variants lead to many different thalassemia genotypes [10]. The various thalassemia genotypes express different symptoms and severity. The clinical manifestations may not be observable in some mild cases unless detected during routine blood tests but the severe forms can even result in failure to thrive [11]. These manifestations are variable ranging from mild hypochromic anemia to moderate hematological disease to severe, lifelong, transfusion-dependent anemia with organ malfunctions [12]. Generally, thalassemia can be broadly classified into α- and β-thalassemia (α- and β-thal) or the rare hereditary persistence of fetal Hb (HPHF), depending on the underlying defective globin chain. Clinically, α- and β-thalassemias may occur in homozygous, intermediate or minor genetic forms and may also form interactions with other Hb variants within the same patient [13].

Diagnosis

Diagnosis of hemoglobinopathies is important, in as much as it can confirm a provisional diagnosis, where they can be identified before symptoms arise as in neonatal screening and most importantly facilitate genetic counseling to prospective parents who might be at-risk of passing on the gene defects to their children [14]. Diagnosis of the condition typically includes analysis of hematological parameters, special Hb tests as well as genetic analysis [6]. Mutation analysis offers effective detection of genotypes that contribute to the understanding of gene interactions that can affect the manifested phenotype [15]. Furthermore, knowledge of family history can also facilitate diagnosis of a hemoglobinopathy. Among the blood parameters of thalassemia are usually low mean corpuscular volume (MCV) or low mean corpuscular Hb (MCH), although not always, while red blood cell (RBC) counts can be normal or slightly elevated. Ferritin levels can be used as differential diagnosis to discriminate between iron deficiency anemia and thalassemia [16].

As not all thalassemia cases present with low MCH or MCV, separations of Hb fractions should be done as a part of diagnosis. There are quite a variety of electrophoretic techniques that can be employed to diagnose hemoglobinopathies and to identify Hb variants. Hemoglobin electrophoresis on cellulose acetate (alkaline pH) or citrate agar (acid pH) also allows identification of Hb variants such as Hb A_1, Hb A_2, Hb F as well as abnormal Hbs, Hb S, Hb C (*HBB*: c.19G>A), Hb D-Punjab (*HBB*: c.364G>C), Hb G-Philadelphia (*HBB*: c.207C>A) and Hb E. Hbs G-Philadelphia and D-Punjab comigrate with Hb S on alkaline gels, and will therefore need to be distinguished by acid gel electrophoresis. However, on acid gels, Hb A and Hb A_2 comigrate with Hb D-Punjab, Hb G-Philadelphia and Hb E, while Hb C, Hb F and Hb S migrate independently [17]. Alternatively, isoelectric focusing is a widely used electrophoretic technique that allows separation of common normal Hbs such as Hb A, Hb F and variants Hb S and Hb C. It is commonly used as it is cost-effective and has good resolution. As some variants are difficult to distinguish, another widely adopted technique is the high performance liquid chromatography (HPLC) that can separate and give a good quantitative measure of Hb fractions in newborns and adults [18]. Hemoglobin variants and thalassemia can be analyzed by using cation exchange columns or reversed phase columns [19]. High performance liquid chromatography can therefore be a secondary screening tool to further support electrophoretic analysis and to confirm identification in cases of those Hb variants that possess the same electrophoretic migration.

Aside from the above mentioned approaches of diagnosis, various molecular techniques have been introduced and employed to facilitate precise diagnosis and contribute to a deeper understanding of the underlying genetic mechanisms behind the diseases [20]. It is critical that individuals who may be asymptomatic and carry these genes are identified so they may be offered genetic counseling on their risks, the nature of the disease and their reproductive choices [21]. Most of the mutations instigating the thalassemia have been characterized and thus, polymerase chain reaction (PCR)-based techniques are widely employed to directly detect mutations in laboratories [22]. Unknown or rare mutations can also be identified by amplification and sequencing methods [23]. Additionally, prenatal genetic screening by methods such as chorionic villus sampling (CVS) and prenatal ultrasound allows identification of affected fetuses. This will facilitate parents' decisions in such cases and also management of pregnancy and intervention in cases of non-immune hydrops fetalis [24]. However, prenatal screening for thalassemia and hemoglobinopathies is not available in Brunei.

Negara Brunei Darussalam

Brunei Darussalam is located in Northwest Borneo of Southeast Asia. Brunei covers an area of 5765 km^2. Brunei is bounded by South China Sea, and Sarawak. Brunei Darussalam has a tropical equatorial climate with a constant high temperature ranging from 23–32 °C, high humidity along with heavy rainfall. According to data from Brunei's Department of Economic Planning and Statistics, the country's population in 2020 was 453,600. In the last reported study for thalassemia and hemoglobinopathies in Brunei, out of 1000 referred patients, it was estimated that 30.0% carried either thalassemia or Hb variants [25]. Although the figure did not represent the population, this sort of prevalence has created the surge in awareness of the disease, improved management and treatments and prevention by genetic counseling.

It has been estimated that carriers of thalassemia and hemoglobinopathies in Brunei is around 5.0% or less. There are currently about 200 patients (inclusive of adults and children) across all four hospitals in the country [N. Yusof, personal communication (unreferenced), February 2020]. The majority of the diagnoses are α-thal trait, β-thal trait, heterozygous Hb E/β-thal, β-thal major (β-TM), β-thal intermedia (β-TI), followed by other less frequent anomalies such as Hb H (β4)

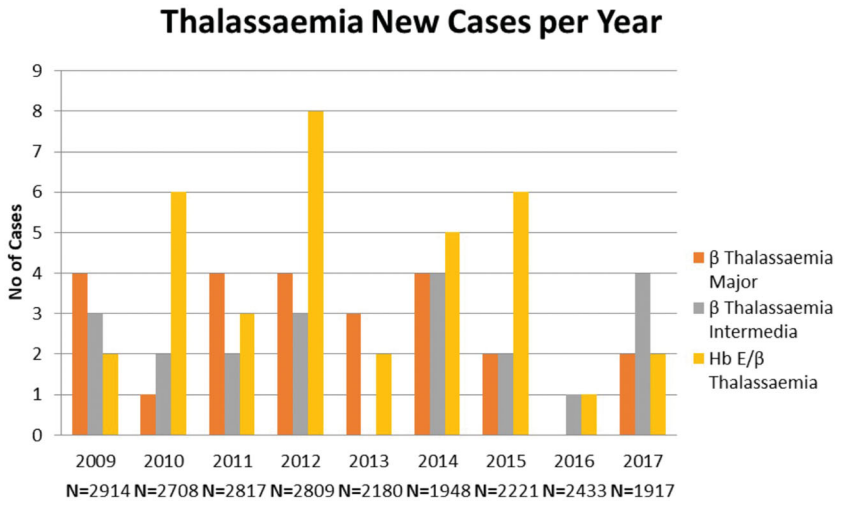

Figure 1. Number of new thalassemia cases per year from 2009 to 2017. Hb E/β-thal cases are reportedly higher than β-TM and β-TI, respectively. *N* is the total number of patients screened for blood parameters in the respective year.

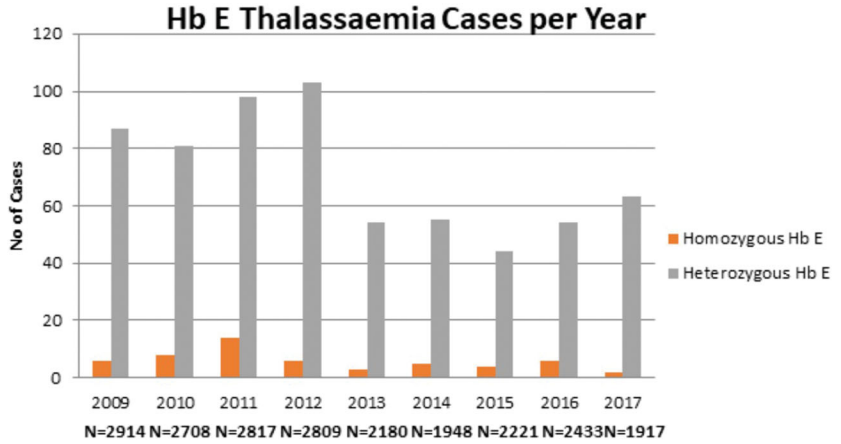

Figure 2. The numbers of Hb E disorders from 2009 to 2017, where the number of heterozygous Hb E disorders is distinctly high each year. N is the total number of patients screened for abnormalities in blood parameters in the respective year.

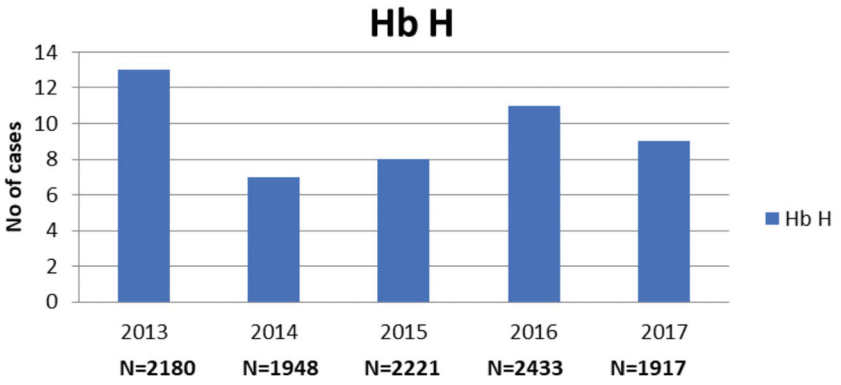

Figure 3. The number of Hb H disorders from 2013 to 2017. *N* is the total number of patients screened for abnormalities in blood parameters in the respective year.

disease, Hb Lepore-Boston-Washington (Hb LBW) ($\delta\beta^{67}$; β^{16}) (NG_000007.3: g.63632_71046del) variant and others.

Data available for the years 2009–2017 were compiled by the Hematology Laboratory at the Raja Isteri Pengiran Anak Saleha Hospital (RIPAS), Bandar Seri Begawan, Brunei Darussalam. By 2017, there were at least 80 confirmed cases of thalassemias and other hemoglobinopathies reported to the hematology laboratory, excluding traits and those without confirmed diagnoses. Figure 1 shows the number of new cases per year of some common thalassemia disorders from 2009 to 2017. The number of Hb E/β-thal cases is shown to be the highest during the respective years.

Figure 2 shows the number of heterozygous Hb E/β-thal cases per year from 2009 to 2017. It is clear that heterozygous Hb E/β-thal cases are prominently high each year ranging from over 40 cases in 2015 to more than 100 cases in

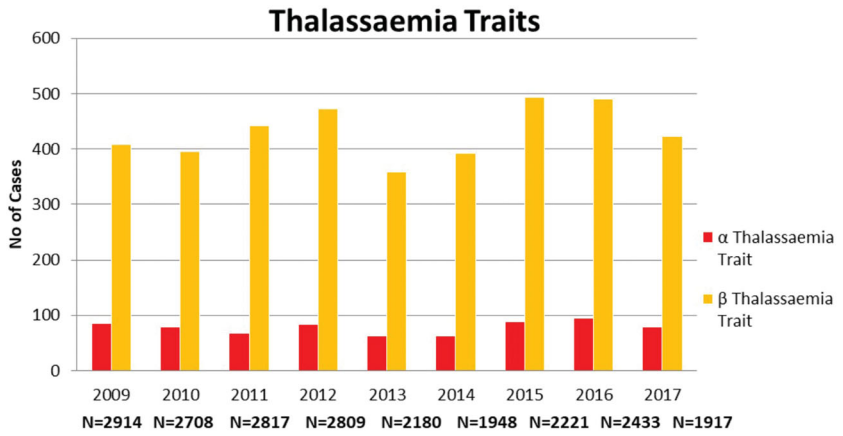

Figure 4. The presumptive number of carriers for α- and β-thalassemias reported at the Hematology Laboratory, RIPAS Hospital from 2009 to 2017. *N* is the total number of patients screened for abnormalities in blood parameters in the respective year.

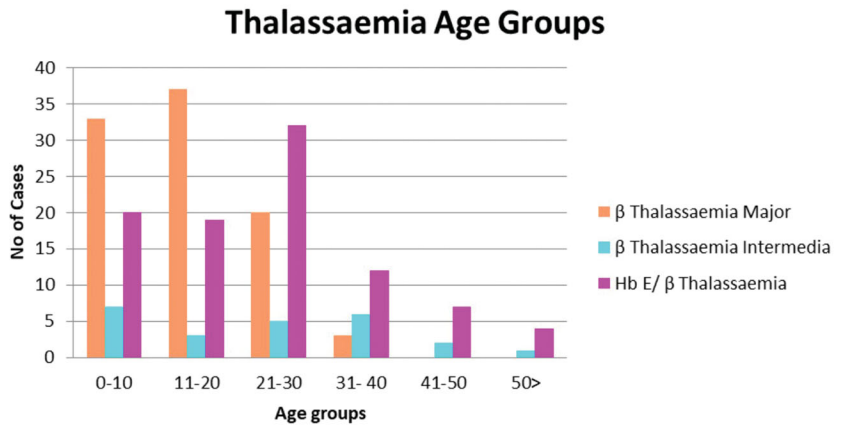

Figure 5. The current number of thalassemia cases within the age groups.

2012. The number of homozygous Hb E cases are distinctively lower.

In Figure 3, the numbers of Hb H disorders in 2013–2017 are shown. The number of cases ranges from seven in 2014 to 13 in 2013, and by 2017 the total number of Hb H disorders in Brunei were 48. Figure 4 shows the α- and β-thal trait cases reported to the Hematology Laboratory, RIPAS Hospital in Brunei Darussalam from 2009 to 2017. The presumptive number of carriers for the β-thal trait is significantly higher than that for the α-thal trait ranging from over 300 and 400 in 2013, 2009, and 2010, respectively, to nearly 500 cases in 2015 and 2016. The α-thal trait numbers are just below 100 per year. The presumptive figures do not represent carrier status for the entire local population of Brunei Darussalam as no population study has been conducted for Brunei.

In Figure 5, there are more cases of β-TM and Hb E/β-thal among the younger age groups (0–40 years old), where β-TM peak at over 35 cases for the 11–20 years old group. Hb E/β-thal peak at over 30 for age group of 21–30 years old. Meanwhile, there are lower numbers of β-TI cases across all the age groups.

In Brunei Darussalam, management of thalassemia patients typically includes investigation, diagnosis, blood transfusion and iron chelation therapy, which are carried out at the Hematology Laboratory and provided by clinicians at the Hemato-Oncology Unit and the Department of Pediatrics, respectively at the main tertiary hospital in the capital city and the other district hospitals. Investigation and diagnosis at the hospital laboratory are primarily by analysis of blood parameters, microscopy, Hb electrophoresis and HPLC. Where primary methods of diagnosis cannot identify the condition and further analysis is required, some patient samples have been sent to overseas laboratory for genotyping analysis. There is no central reporting system of cases of thalassemia. Thus, the establishment of a thalassemia registry encompassing relevant data on patients and patient outcomes, is vital as a tool for monitoring and improving quality of healthcare for patients [26]. With this information and data collated locally and overseas, cross-comparison of data can help with development of carrier detection and genetic counseling services to create control strategies to reduce the prevalence of thalassemias in the population. Awareness of the condition by research at the university level and public education and campaigns in the Brunei community will contribute to a long-term strategy in improving prevalence of the disease. This review is a first step in collating available data in understanding the status of thalassemias and hemoglobinopathies in Brunei Darussalam and moving forward for genotyping and the National Thalassemia Registry for the country.

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Disclosure statement

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