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Practical Outcomes of Hemoglobin Electrophoresis Results Evaluation in Estonia

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Summary

Objectives. Hemoglobinopathies (HbP) are particularly frequent in the Mediterranean countries, in the Middle East, in both West and North Africa, as well as in India and other Asian countries. Multi-ethnic migrations however, are now affecting the epidemiology of hemoglobinopathies. Non-endemic countries such as Estonia, Latvia, Lithuania are affected by thalassemia-related problems due to increasing migration from endemic to non-endemic areas. The prevalence of HbP in the Baltic Sea region is unknown. In non-endemic countries physicians need to know when to suspect and how to diagnose hemoglobinopathies.

The aim of the current study was to assess hemoglobin electrophoresis results retrospectively and define the necessary steps in the diagnostic workflow of HbP.

Methods. The current study is a retrospective analysis of 339 samples submitted for hemoglobin electrophoresis evaluation between 2011 and 2018 at the laboratory of North Estonia Medical Centre. The subjects were divided into three groups based on the HbA2 reference values (2.5–3.5%) used in our laboratory: group-1 (<2.5%), group-2 (2.5–3.5%), group-3 (>3.5%).

Results. 65 cases of hemoglobinopathy were found. As a result of this study, a standardised Information Sheet (questionnaire) was developed to be used prior to ordering the hemoglobin electrophoresis via the laboratory information system (LIS). Also, a diagnostic workflow was developed and implemented for routine use.

Conclusions. In recent decades the identification of hemoglobinopathies in non-endemic countries has become very important and requires focused attention. Interpretation of results is a complex process involving multi-step testing and multidisciplinary approach. In this context, it is important to routinely collect information necessary for interpretation and to establish standardised workflows.

Keywords: hemoglobinopathies, thalassemia, sickle cell, hemoglobin electrophoresis.

BACKGROUND

Hemoglobinopathy (HbP) conditions are hemoglobin disorders caused by structural abnormalities and/or alteration in the amount of globin chains. Approximately 7% of the global population carries the abnormal hemoglobin gene [1]. Thalassemia is the most common heredity type of HbP. Despite originally being endemic to large regions in Asia, Africa and Mediterranean, it is now wide-spread globally due to international migration [1]. Non-endemic countries such as Estonia, Latvia, Lithuania are affected by thalassemia-related problems due to increasing migration from endemic to non-endemic areas. The prevalence of HbP in the Baltic Sea region is un-

Table 1. **Hematological data in the study subjects**

Parameter	Group-1 (n=109)	Group-2 (n=177)	Group-3 (n=53)	*P**	*P***	*P****
RBC (10^{12}/L)	4.7 (4.2–5.4)	4.7 (4.1–5.2)	5.7 (5.4–6.4)	0.317	<0.05	<0.05
MCV (fL)	75.2 (68.6–81.4)	84.4 (77.1–89.5)	61.2 (58.5–63.2)	<0.05	<0.05	<0.05
MCH (pg)	23.5 (21.1–27.0)	28.3 (25.9–29.9)	19.9 (18.9–20.6)	<0.05	<0.05	<0.05
HbA2 (%)	2.2 (2.0–2.3)	2.7 (2.6–2.8)	5.3 (5.0–5.7)	<0.05	<0.05	<0.05

*Comparison between group-1 and group-2
** Comparison between group-2 and group-3
***Comparison between group-1 and group-3

known. In Estonia, the first beta-thalassemia incidence was reported in 2006 [2]. In Lithuania, a novel variant hemoglobin was reported in 2016 [3] and in 2019 a case of beta-thalassemia during pregnancy was discussed and a review of etiopathogenesis, clinics and diagnostics was conducted [4].

It is increasingly important for the physicians of non-endemic countries to know when to suspect and how to diagnose HbP. To that end, the hemoglobin electrophoresis has been implemented in North Estonia Medical Centre since 2011.

THE AIM

The aim of the current study was to assess hemoglobin electrophoresis results retrospectively and define the necessary steps in the diagnostic workflow of HbP.

METHODS

This study was designed as a retrospective study based on data from the laboratory information system (LIS). We analyzed the samples of all 339 individuals (45.9% male, 3–98 years) subjected for hemoglobin electrophoresis evaluation in Estonia between 2011 and 2018 at the laboratory of North Estonia Medical Centre.

Peripheral venous blood specimens were collected into K2-EDTA tubes (BD Vacutainer, BD Diagnostics, Plymouth, UK) for complete blood count (CBC) and hemoglobin electrophoresis. Hemoglobin (Hb), red blood cell (RBC) count, mean cell volume (MCV), mean cell hemoglobin (MCH) were provided by the Sysmex-XE 5000 (Sysmex Corporation, Kobe, Japan). Morphology of peripheral blood was evaluated. The hemoglobin electrophoresis tests were performed on MiniCap Capillary Electrophoresis System according to manufacturer's instructions (MiniCap, Sebia, France).

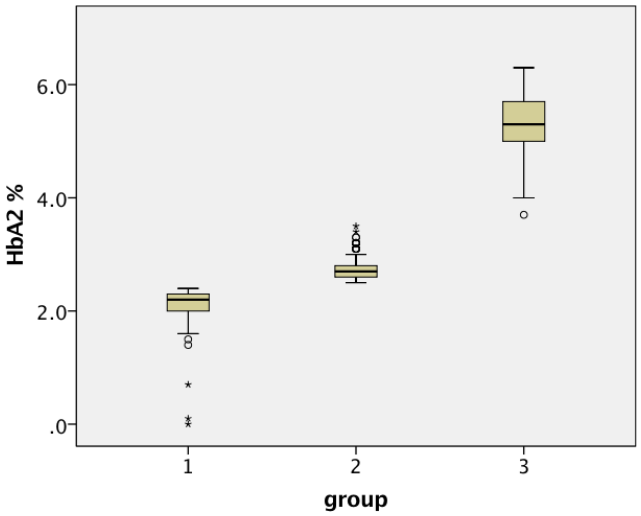


Fig. 1. **Differences of HbA2 for all study groups**
Median (bold line), upper quartile (upper edge of gray box), lower quartile (lower edge of gray box), maximum (upper bar), minimum (lower bar) and outlier (circle) values of HbA2 (%) are given.

Information relevant to the analysis (such as iron status) was queried via LIS. Additional patient data was collected via communication with clinicians when the data was incomplete.

The subjects were divided into three groups based on the reference

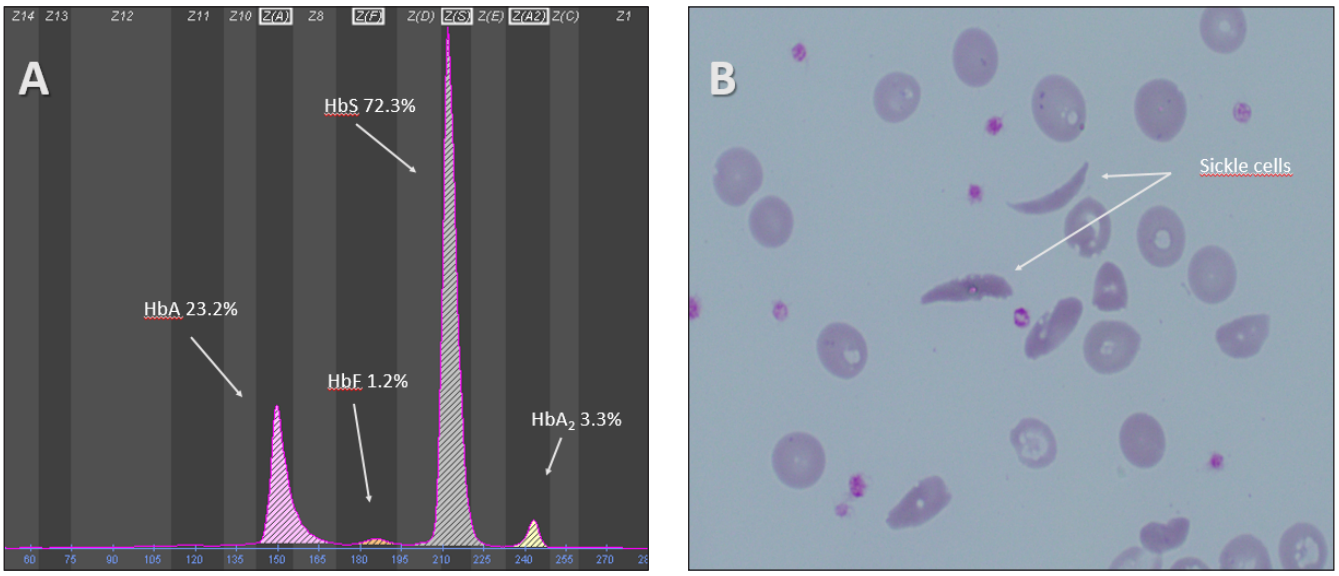


Fig. 2. **Case of sickle cell disease**
A – peaks of Hb variants by Capillary Electrophoresis (Sebia), B – peripheral blood smear with sickle cells

Table 2. **The Information Sheet (questionnaire) to be filled prior to the ordering of hemoglobin electrophoresis**

Information required for interpretation		
Results of laboratory tests	***Clinical suspicion of hemoglobinopathy***	***Patient data***
• CBC with automated differential, RET and blood smear evaluation • Iron, ferritin, transferrin, transferrin saturation, folic acid and vitamin B12	• Unexplained microcytosis • Unexplained hemolytic anemia • Unexplained cyanosis • Unexplained polycythemia	• Patient's age • Geographical /ethnic origin • Medical family history • Blood transfusion in last 3 months? Yes/No • Pregnancy? Yes/No

values [5] for HbA2 (2.5–3.5%): group-1 (<2.5%), group-2 (2.5–3.5%), group-3 (>3.5%).

Statistical analysis was done using the SPSS software 21.0 (IBM). The results are expressed as median (25th–75th percentile). The difference between variables was tested using the Mann-Whitney test. The p value < 0.05 was considered statistically significant.

The study was performed according to the Declaration of Helsinki and was approved with National Institute for Health Development Ethics Committee decision number 2602.

RESULTS AND DISCUSSION

The present study investigated 339 hemoglobin electrophoresis results measured from 2011 to 2018. The results are summarized in Table 1. The RBC were higher in group-3 than in group-1 and group-2 ($p<0.05$), difference between group-1 and group-2 was insignificant ($p=0.317$). The MCV and MCH were lower in group-1 and group-3 than in group-2 ($p<0.05$). The HbA2 was lower in group-1 (2.2%, varied between 1.0% and 2.4%) than in group-2 (2.7%, varied between 2.5% and 3.5%) and group-3 (5.3%, varied between 3.7% and 6.3%), which is illustrated in Fig. 1. The differences between the groups were statistically significant ($p<0.05$).

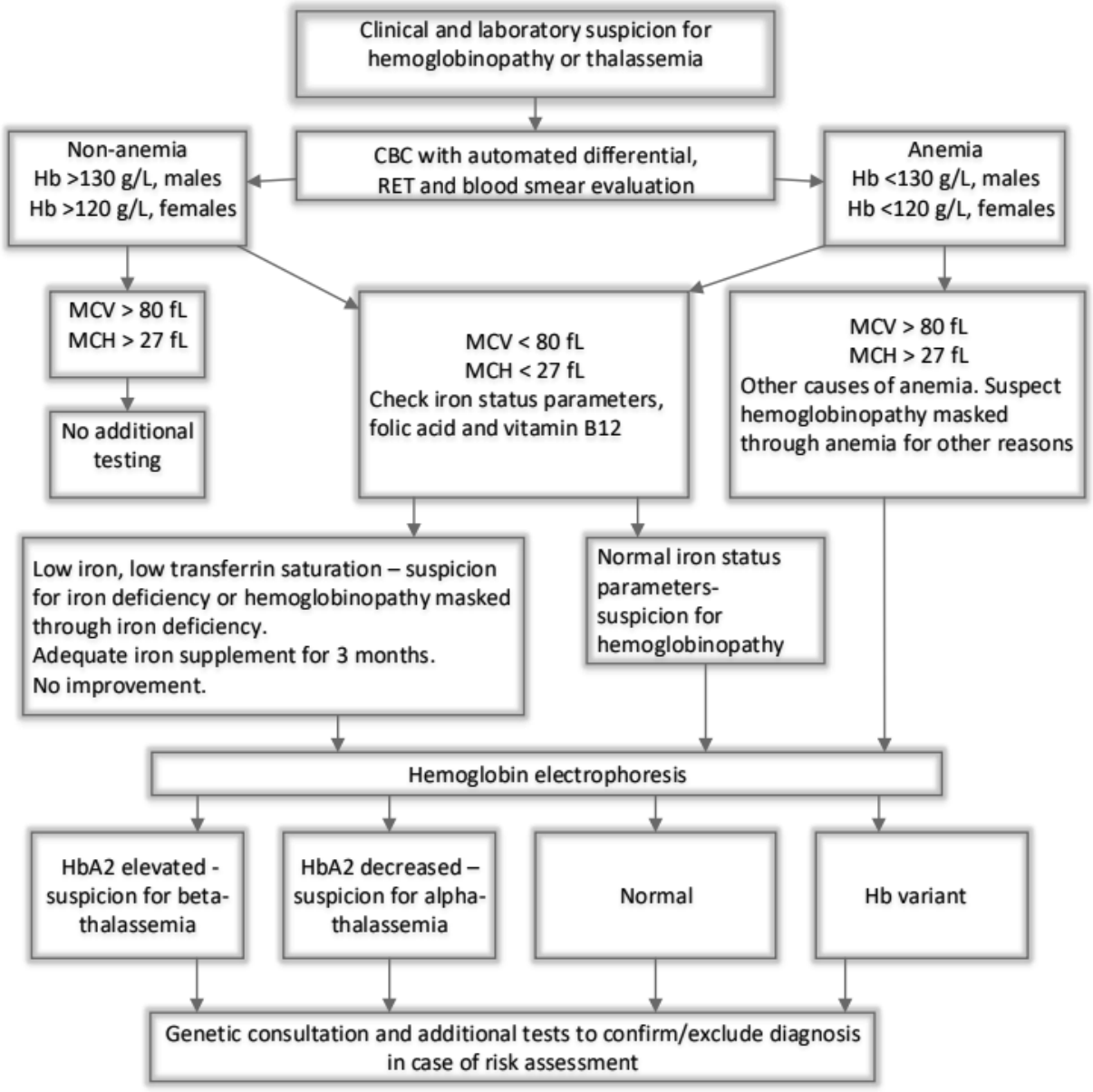


Fig. 3. **The diagnostic workflow of suspicion for hemoglobinopathy or thalassemia**

The results of electrophoresis were interpreted according to the manufacturers (MiniCap, Sebia, France) recommendations [5].

We found that in group-1, HbF was increased in 8 cases (ranging from 1.0% to 83.3%), Hb Lepore (12.9%), HbS variant (32.4%) and HbD/HbS variant (35.9%) was detected in one case each. HbA2 levels <2.5% is essential for a diagnosis of -thalassemia [6, 7]. It should be reminded that lower values of HbA2 might be found in sideroblastic anemia [8], severe iron deficiency [9], beta-delta-thalassemia, alpha-chain variants, delta-chain variants, HbH disease, some forms of hereditary persistence of fetal Hb, Hb Lepore and erythroleukemia [6].

In group-2, HbF was increased in 13 cases (ranging from 1.0% to 29.6%), HbS variant was detected in 4 cases (ranging from 38.0% to 82.4%) and in one case, sickle cells were found by microscopic investigation suggestive of sickle cell disease (Fig. 2). HbE variant was identified in 2 cases (ranging from 36.2% to 60.9%) and Hb Lepore once (10.0%).

The normal value of HbF 2 years after birth is <1% [10]. In group-1 and group-2 HbF >1% was found in 7% of cases. Mostly, elevated HbF level is associated with pregnancy, delta-beta-thalassemia, bone marrow malignancies, aplastic anemia, erythropoietic stress, Fanconi anemia, hereditary persistence of HbF, BCL11A mutations and HBS1L-MYB mutations [11].

In group-3, HbF was increased in 14 patients (ranging from 1.0% to 14.4%), HbS variant (28.1%) and HbE variant (58.7%) were detected in one case each. Patients with HbA2 level >3.5%, MCV <80 fL, MCH <27 pg were classified as beta-thalassemia. Despite elevated HbA2 is one of the indicators of beta-thalassemia, the migration of some hemoglobin variants with HbA2 can result in the overestimation in the quantification of HbA2 [10]. Mosca A, et al. [6] report, that several subject-related factors, such as hyperthyroidism, megaloblastic anemia, some unstable Hb variants, hypertrophic osteoarthropathy, might increase HbA2.

It should be noted that in group-2 90.4% of samples displayed had result patterns that could be interpreted as normal. However, normal values of HbA2 may present in cases with iron deficiency, alpha-thalassemia trait, 'normal-HbA2' beta-thalassemia, delta-beta-thalassemia or a combination of these [12]. In current study, iron status results were only available for 62% cases, and other important supplementary information such as the reason for referral, demographic data, the clinical background, recent transfusion history, and medication was not provided in LIS. Therefore, an accurate interpretation of hemoglobin electrophoresis results was difficult and required the additional communication with clinicians. As a result of these communications, it was revealed that in 14 cases the patients had received blood transfusion prior to the blood collection for analysis. As the blood transfusion prior to the hemoglobin electrophoresis may make interpretation difficult, a need for better information collection was made evident.

The newly developed Information Sheet (questionnaire) to be implemented prior ordering the hemoglobin electrophoresis via LIS can be found in Table 2. The Diagnostic Workflow (Fig. 3) was developed and implemented for routine use.

CONCLUSIONS

Even though Estonia is a non-endemic country with a population of 1.3 million, 65 cases of hemoglobinopathy have been found in recent years. This illustrates that the awareness about hemoglobinopathies is increasingly important in non-endemic countries and requires focused attention. Interpretation of results is a complex process involving multi-step testing and multidisciplinary approach. In this context, it is important to routinely collect information necessary for interpretation (such as in the developed Information Sheet) and to establish standardised workflows (Diagnostic Workflow).

Disclosure of Interests

Karit Krause is an employee of Mediq Estonia OÜ (distributor of Sebia in Estonia). The other authors have no conflict of interest to declare.

Author contributions

All the authors have accepted responsibility for the entire content of this submitted manuscript and approved the submission.

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Santrauka

HEMOGLOBINO ELEKTROFOREZĖS DUOMENŲ VERTINIMAS ESTIJOJE: PRAKTINIAI REZULTATAI

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Įvadas. Hemoglobinopatijos (HbP) yra ypač paplitusios Vidužemio jūros regione, Viduriniuose Rytuose, Vakarų ir Šiaurės Afrikoje, taip pat Indijoje bei kitose Azijos šalyse. Dabartinė daugiatautė migracija keičia hemoglobinopatijų epidemiologiją. Neendeminės šalys, tokios kaip Estija, Latvija, Lietuva, susiduria su talasemijos atvejais dėl didėjančios migracijos iš endeminių zonų į neendemines. HbP paplitimas Baltijos jūros regione nežinomas. Gydytojams neendeminėse šalyse svarbu žinoti, kaip įtarti ir diagnozuoti hemoglobinopatijas.

Šio tyrimo tikslas buvo įvertinti hemoglobino elektroforezės rezultatus retrospektyviai ir nustatyti reikalingus HbP diagnostikos sekos etapus.

Metodai. Šis tyrimas yra 339 mėginių, pateiktų hemoglobino elektroforezės analizei įvertinti Šiaurės Estijos medicinos centro laboratorijai 2011–2018 m., retrospektyvioji analizė. Tiriamieji buvo suskirstyti į tris grupes pagal mūsų laboratorijoje naudojamas HbA2 pamatinius biologinių verčių intervalus (2,5–3,5 %): 1 grupė (< 2,5 %), 2 grupė (2,5–3,5 %), 3 grupė (> 3,5 %).

Rezultatai. Nustatyti 65 hemoglobinopatijos atvejai. Po šio tyrimo buvo sukurta standartizuota informacinė forma (klausimynas), naudojama prieš užsakant hemoglobino elektroforezę per laboratorijos informacinę sistemą (LIS). Taip pat buvo sukurta ir įdiegta diagnostikos eigos seka kasdieniniam naudojimui.

Išvados. Nustatyti hemoglobinopatijas neendeminėse šalyse pastaraisiais dešimtmečiais tapo itin svarbu, tam reikia išskirtinio laboratorinės medicinos gydytojų ir klinicistų dėmesio bei glaudaus jų bendradarbiavimo. Dėl šios priežasties svarbu reguliariai rinkti ir interpretuoti reikalingą informaciją bei nustatyti diagnostikos eigos seką.

Reikšminiai žodžiai: hemoglobinopatijos, talasemija, pjautuvinės formos ląstelės, hemoglobino elektroforezė.

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