

"MINOR" HEMOGLOBINOPATHIES: A RISK FACTOR FOR ASTHMA*

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SUMMARY:

■ **Background:** Thalassemia, sickle cell disease and other hemoglobinopathies are not rare in Portugal and European Mediterranean area. Homozygotic patients present major hematological disease but heterozygotic minor asymptomatic forms are frequent, mainly thalassemia minor and sickle cell trait. In these cases mycrocytosis with decrease of red cell volume and mean corpuscular volume or abnormal rigid erythrocytes are found and can lead to hemorheologic disturbances. The purpose of this study is to evaluate the incidence of asthma in hemoglobinopatic patients allergic to house dust mites.

■ **Method:** From 4.000 patients seen in the last 5 years in an out-patient allergy clinic, 63 cases of hemoglobinopathies have been confirmed by red cell count, hemoglobin electrophoresis, assays of hemoglobin A2, Fc, and S, and sickle cell test. All these patients had allergic disease characterized by clinical history, skin prick test to aeroallergens total and specific IgE (RAST-CAP-FEIA) and respiratory function evaluation.

■ **Results:** 66 Hemoglobinopathies: Betathalassemia 61 cases, Betadelta thalassemia 2, sickle cell trait 2, Hemoglobin C, 1. 57 patients have respiratory allergy, rhinitis in 14 cases of thalassemia and 1 of hemoglobin C, asthma with or without rhinitis in 41 cases of thalassemia and 1 case of sickle cell trait, the other 6 cutaneous allergy. Therefore asthma was present in 75,0% of the respiratory allergic patients and rhinitis only in 25,0%. In contrast in a control group of 491 respiratory allergic patients without hemoglobinopathies, 57% has asthma and 43% only rhinitis.

■ **Conclusion:** The prevalence of asthma is higher in thalassemia minor and sickle cell trait ($p < 0.05$ Square chi test). Hemorheological changes probably a greater rigidity of red blood cells in capillary bed can contribute to changes in bronchial circulation and bronchial hypereactivity. Detection of hemoglobinopathies must be done in asthmatic patients with slight anemia or mycrocytosis.

Key-words: Asthma - Hemoglobinopathies

Allergic diseases as well known are very common in European populations. In Mediterranean area and all Southern Europe hemoglobinopathies are not rare attaining in certain countries and regions values as high as 15%-20% of subjects, mainly heterozygotes (Bernard, Ruffie, Galacteros, M.L.Palma-Carlos, 1982, 1983). Hemoglobinopathies are hereditary diseases presenting different degrees of severity. Homozygotic hemoglobinopathies are for the most current forms, sickle cell disease and thalassemia very severe diseases, with a lethal evolution in absence of bone-marrow transplant. In contrast heterozygotic forms are usually assymptomatic and in most cases even without anemia and only laboratory studies can detect it (Benz, Beutler, Embury, Lehman 1974, 1976, Rodgers, Weatherall, 1972, 1977, 1995).

Human adult hemoglobin is a heterogenous mixture of proteins consisting of a major component, hemoglobin A and a minor component hemoglobin A2. All the normal human hemoglobin in children and adults have one pair of Alpha chains combined with Beta chains in hemoglobin A with Delta chains in hemoglobin A2 and with Gama chains in hemoglobin F (Benz, Efremov, Lehman, Miale, Nechelles, Weatherall 1972, 1977, 1995 ; Wintrobe).

Thalassemias are usually classified in Alpha, Beta and Delta Beta (Wheaterall 1972, 1977, 1995).

Beta thalassemia more common in Europe is extremely heterogenous at the molecular level mainly by deletions of the Beta globin gene and non deletional mutations that may affect the transcription processing or transmission of Beta globin messenger RNA (Weatherall 1995 ; Wintrobe).

Delta-Beta thalassemias are due to deletion that remove or inactivate only the delta and Beta genes (Galacteros, Weatherall 1995 ; Wintrobe).

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Double heterozygosity for two hemoglobinopathies for instance sickle-cell disease and thalassemia also causes a very severe disease (*Benz, Galacteros, Nechelles, Weatherall 1995*).

Thalassemia major and sickle cell disease, the most common forms of hemoglobinopathies, present a very severe disease and allergy, if present, is secondary. These patients can however after bone marrow transplant from an allergic donor presenting allergic symptoms.

Thalassemia minor, sickle cell trait or heterozygosity for hemoglobin C are generally considered as mild forms of hemoglobinopathies without clinical symptoms or for some cases of thalassemia only a slight anemia or mycrocytic polyglobulia (*Benz, Galacteros, Nechelles, Weatherall 1995 ; Wintrobe*).

In Portugal different forms of thalassemia, sickle cell disease or trait and other hemoglobinopathies are not uncommon, mainly in certain coastal zones and the southern part of the country (*Palma-Carlos, ML. 1982, 1983*).

Thalassemia in Southern Europe must be due to miscegenation in the populations coming from Mediterranean area, in Iberian Peninsula specially from North Africa, the migrations in pre-historic period and the contacts with greeks, phoenicians, carthaginenses, romans who settled in Hispania and again populations from North Africa after Muslim invasion can explain this miscegenation.

Other hemoglobinopathies are also due to the portuguese exchanges with Africa and Asia from the XVth century. Portuguese medical doctors have noted in the XVII and XVIII century that the populations of Congo River area were partially resistant to malaria. Therefore a plan of re-population of the areas in Southern Portugal where malaria was endemic was applied in the middle of the XVIII century.

We know nowadays that malaria resistant subjects were the sickle cell trait patients. Slaves from Congo area were brought to Portugal liberated organized in "Confradys" and given fields to cultivate.

Today after 250 years of miscegenation with local white population the only and single remaining trait of african origin is hemoglobin S.

In previous studies our group has detected a large number of hemoglobinopathies (271) in Portugal including 166 thalassemias (table 1) and 105 abnormal hemoglobins (table 2) (*Palma-Carlos ML 1982, 1983*).

166 THALASSEMIAS

BETA MAJOR	2
BETA DELTA MAJOR	1
BETA LEPORE	1
BETA MINOR	141
BETA DELTA MINOR	3
BETA-HbS	18

Table 1.

105 ABNORMAL HEMOGLOBINS

Hb SS	31
Hb SA	56
HbC	3
Hb Köln	3
Hb D Punjab	1
Hb S Baltimore	1
Hb Castille	1
Unstable non yet identified hemoglobins	9

Table 2.

We reported in 1989 and 1990 the association of hemoglobinopathies with allergy (*Palma-Carlos ML 1989, Palma-Carlos AG. 1990*) Due to the high incidence of both diseases in Portugal this association was to be expected. In the last few years an increased number of hemoglobinopathies has been diagnosed during the general check-up of allergic patients in our clinical outpatient Center. The analysis of this larger serie has suggested that in allergic patients with minor hemoglobinopathies - thalassemia minor, sickle cell trait and hemoglobin C trait, the incidence of asthma in respiratory allergic patients was increased in report to the allergic populations with allergy to house dust mites (*Costa et Palma-Carlos 2001, Palma-Carlos et Palma-Carlos 2004*). Even if the concept of comon allergic rhinitis asthma syndrome or unified airways disease has been

largely accepted in the last few years (*Palma-Carlos et al 2001*) the occurrence of risk factors increasing the incidence of asthma cannot be denied. An analysis of clinical presentation of respiratory allergy in patients with minor hemoglobinopathies will be presented.

The general methods for the diagnostic of hemoglobinopathies are quoted in table 3. These methods have been applied in our study.

DIAGNOSIS OF HEMOGLOBINOPATHIES

- HEMOGLOBIN A2 INCREASED IN BETA THALASSEMIA (SOME CASES WITH NORMAL LEVEL OF HbA2)
- HEMOGLOBIN F INCREASED IN DELTA BETA THALASSEMIA
- HbS INCREASED AND POSITIVE SICKLE CELL TEST IN SICKLE CELL TRAIT
- HbC=HbA2 IN ELECTROPHORESIS WITH SICKLE CELL TRAIT NEGATIVE AND AGAR GELL ELECTROPHORESIS POSITIVE

Table 3.

However, there are forms of Beta thalassemia in which heterozygotes have normal hemoglobin A2 levels. Two main classes of normal hemoglobin A2 Beta thalassemia are described: Type-1 genuine form of beta thalassemia while type 2 is heterogenous with at least some cases representing a compound heterozygous state for Beta thalassemia and delta thalassemia (*Weatherall 1995*). In these cases a decrease of mean corpuscular volume (MCV) between 70 and 75 and mainly a dissociation between mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), the first decreased (20-25 pg) the second approaching normal values (32-35 g/dl) suggests the possibility of thalassemia (*Klee, Weatherall 1995*).

In thalassemia mean MCH is reduced well as MCV, while MCHC may be normal or only slightly reduced (*Nechelles, Wintrobe*).

Therefore the anemia is more microcytic that hypochromic. The most helpful of red cell indice in Thalassemia trait and heterozygotic B Thalassemia is the MCH which is low on all cases (*Weatherall, Klee*).

Increased level HbA-2 is only found in thalassemia and some HbS and HbC hemozygotes and heterozygotes. This useful disgnostic feature is lost in presence of iron deficiency but is corrected after iron therapy (*Wintrobe, Weatherall*).

Haemoglobin C migration in cellulose acetate electrophoresis is very close to Hb-A2. However it could be separated by agar gel electrophoresis (*Miale, Nechelles, Weatherall, Wintrobe*).

MATERIAL

From a series of 4 000 allergic patients seen in the last 5 years a systematic study of abnormal hemoglobins has been done in all subjects presenting a slight poly-

globulia, microcytosis (MCV less than 75 PG) or a dissociation between mean corpuscular hemoglobin (MCH) and mean concentration of corpuscular hemoglobin with the first (MCH) equal or less than 26 and the second in the normal range (*Beutler, Klee, Miale, Rodgers, Wintrobe*). A group of 491 patients seen in the same period presenting also allergy to house dust mites but with a completely normal hemogram has been used as control.

METHODS

In all the suspected cases hemoglobin electrophoresis, research of alkali resistant hemoglobin, assay of hemoglobulin A2 by chromomatography, sickle cell test have been done. In suspected cases agar gel electrophoresis for hemoglobin C has been applied (*Miale, Weatherall, Lehman, Efremov*).

Sideremia and ferritin have been dosed in all patients to exclude iron deficiency and an eventual decrease of hemoglobin A2 maskering thalassemia in these cases (*Benz, Lehman, Efremov, Wheaterall*).

Allergic disease (rhinitis and asthma) have been confirmed by clinical history, skin prick tests to relevant allergens, total and specific IgE respiratory function evaluation and thorax X Rays and sinus CT Scans.

RESULTS

66 hemoglobinopathies have been diagnosed corresponding to 1,55% of the patients observed. In only 3 (4,7%) of these patients hemoglobinopathy has been previously diagnosed. This group comprises 61 cases of Beta thalassemia minor, 2 cases of delta Beta thalassemia minor, 2 cases of sicke-cell trait and 1 case of hemoglobin C trait (table 4). From these 66 cases, 60 presented respiratory allergy and only 6 cutaneous allergy (5 urticarias, 1 atopic eczema dermatitis syndrome (table 5).

From the 60 cases of hemoglobinopathies presenting respiratory allergy (table 6) asthma with or without rhinitis was present in 45 (75,0%), 41 thalassemias minor and 1 sickle cell trait.

Rhinitis without asthma was present only in 15 cases (25,0%), 14 thalassemias minor and 1 case of heterozygotic hemoglobin C. Comparing these results with the distribution of asthma (57%) and - isolated rhinitis (43%) in control group (table 7) a striking and statistically significant difference is found ($p<0,05$). The incidence of asthma is therefore increased in minor hemoglobinopathies.

HEMOGLOBINOPATHIES AND ALLERGY – 66 CASES (1,65%)

BETA THALASSEMIAS MINOR	61
DELTA BETA THALASSEMIA MINOR	2
SICKLE CELL TRAIT	2
HEMOGLOBIN C TRAIT	1

Table 4.

HEMOGLOBINOPATHIES AND ALLERGY ALLERGIC DISEASES – 66 CASES

URTICARIA – 5 CASES
ATOPIC ECZEMA DERMATITIS SYNDROME– 1 CASE
RESPIRATORY ALLERGY – 60 CASES

Table 5.

HEMOGLOBINOPATHIES AND ALLERGY
RESPIRATORY ALLERGY – 60 CASES
ASTHMA WITH OR WITHOUT RHINITIS – 45 CASES –
75,0%
44 THALASSEMIAS, 1 SICKLE CELL TRAIT
RHINITIS ONLY – 15 CASES – 25,0%
14 THALASSEMIAS, 1 HbC

Table 6.

HEMOGLOBINOPATHIES RHINITIS AND ASTHMA

HEMOGLOBINOPATHIES :	
asthma	75,0%
(60 ALLERGIC PATIENTS):	
rhinitis	25,0%
CONTROL GROUP	
(491 ALLERGIC PATIENTS):	
asthma	57,0%
rhinitis	43,0%
X ² p<0,01	
ALL PATIENTS ALLERGIC TO MITES	

Table 7.

DISCUSSION

Diagnosis of minor hemoglobinopathies is easily missed. In our series only in less than 5% of the patients (3 in 66 – 4,5%) the hemoglobinopathy was been diagnosed by GP Pediatricians or Internists and in 2 cases linked to a family history. Most cases of thalassemia minor are considered iron deficiency anemias and treated with iron without success but with serious risk of iron surcharge (*Benz, Weatherall 1995*). In countries where hemoglobinopathies are common a more careful analysis of current blood analysis is mandatory. A decrease of MCV and the dissociation of the 2 RBC indexes, MCH (decreased) and MCHC (around normal) suggest the search for quantitative (thalassemia) or qualitative (Hemoglobins S and C) disturbances of hemoglobin synthesis (*Bent, Effremov, Lehman, Klee, Miale, Weatherall, Wintrobe*).

The greater incidence of asthma in allergic patients with minor hemoglobinopathies has been, as long as we know, first reported by our group (*Costa, Palma-Carlos and Palma-Carlos 2001, Palma-Carlos, Palma-Carlos and Costa, 2004*).

In current practice allergologists and chest physicians don't know about hemoglobinopathies and hematologists, pediatricians, internists and GPs are generally focussed on the main disease, not in association of traits with another clinical presentation.

A larger view of Medicine from those coming from Internal Medicine is necessary to cover both chapters of Medicine and suggest possible connections between hemoglobinopathies and asthma.

The mechanism explaining this probable risk factor for asthma are not yet fully explained but hemorheological factors or genetic factors can be suggested (*Palma-Carlos and Palma-Carlos 2004, Borrich 2004*).

Hemorheological disturbances or a genetic association are two possible explanations of these findings.

In thalassemia minor red blood cells are microcytic, with a decreased osmotic fragility more rigid than normal erythrocytes with anisocytosis presenting in a large number the characteristic target cells (*Benz, Miale, Rodgers, Wheaterall, Wintrobe*). A polyglobulia is usually associated. In these conditions the circulation in bronchial capillary bed could be disturbed and an increase in capillary rigidity contributes to ventilatory disturbances associated with asthma.

Generalized intravascular sickling has been reported with respiratory insufficiency and concomitant desatu-

ration of the arterial blood (*Nechelles*) in sickle cell trait. Even if that such cases are rare this possibility must be considered as a risk factor in asthmatic patients.

In sickle cell trait distorted sickle cells are present with secondary celleration in red cell metabolism and membrane structure and function and decrease of blood flow in microvasculature with rheologic disturbances secondary to partial sickling process (*Beutler, Embury*).

Red cells containing a high proportion of haemoglobin C are more rigid than normal and unable to pass through small pores (*Weatherall 1977, 1995*).

Genetic association is other possibility to consider and a linkage between one of the genes for asthma allergy and atopy and the genes of hemoglobinopathies can't not be excluded.

Hemoglobin alpha chains are coded in alpha gene cluster located in chromosome 16 and Beta chains in the Beta gene cluster in the short arm of chromosome 11 (*Benz, Galacteros, Lehman, Weatherall 1995*).

For chromosome 11 markers for atopy, allergy and asthma have only been reported in the long arm. The genetic linkage 11q13 was first reported in last 90's by *Cookson and Hill*. This linkage was preferentially active in maternally derived alleles (*Cookson, Hill, Holgate*). The gene encoding the Beta subunit of the high affinity IgE receptor was proposed as candidate gene (*Holgate*). Two announced substitutions within exon 6 were reported less 181 and less 183, highly predictive of atopy when inherited maternally (*Holgate*). A substitution of glutamic acid for glycine at residue 237 has been associated with bronchial hyperresponsiveness however with a variable and sometimes weak association with bronchial hyperresponsiveness.

Therefore a linkage between the genetic markers for Beta thalassemia and asthma seems not probable but other genetic changes for instance crossing over can be involved.

The main points to clarify in this context are the evaluation of lung resistance in asthmatic patients with hemoglobinopathies, the hemorheologic properties of red blood cells in these same subjects and also of microcirculation in capillary bed. Genetic detailed studies could perhaps detect some genetic event or association between the genes for hemoglobinopathies and asthma even if the actual data don't point to a linkage. In some cases of more serious asthma genetic counselling could be suggested.

Finally aside of the actual treatment of asthma with bronchodilators and anti-inflammatory drugs it is possible to try, if the disease is more severe drug acting in the rigidity of erythrocytes. A folic acid supplement is currently used in most cases of thalassemia minor with polyglobulia to cover the increased erythropoiesis. However folic acid don't act in hemorheologic properties of red blood cells.

Around 25 years ago piracetam has been proposed as an additional drug to treat sickle cell disease. However the clinical and laboratory results have not been conclusive (*Palma-Carlos et Carmo 1981*).

Piracetam enter red blood cells easily (*Lleyssen et al*) and seems to act on hemorheological properties of erythrocytes, is free of side effects and could perhaps been tried at least in the association sickle cell trait - asthma.

References

1. Benz J.-R., EJ. - Hemoglobinopathies pg 666-674 in *Harrison's Principles of Internal Medicine Braunwald E., Hauser SL., Fauci AS., Longo DL., Kasper DL., Jameson JL. (eds) McGraw Hill, New York 2001.*
2. Bernard J., Ruffié J. - *Hématologie Géographique*. Masson, 1966, Paris.
3. Blumenthal M.-N., Björkstén B. - *Genetics of allergy and Asthma*. Marcel Dekker, New York 1997.
4. Borish L. - Discussion of oral presentations, *American College of Allergy, Asthma and Allergy, Boston, 2004.*
5. Beutler E. - The sickle cell diseases and related disorders, pg 616-649 in *Hematology, Beutler E., Lichtman M.-A., Coller B.-S., Kipps T. (eds) McGraw-Hill 1995 New York*
6. Cookson WOCM., Palmer L.-J. - Investigating the asthma phenotype. *Clin Exp Allergy* 1998;28 (suppl.1) 88-89.
7. Costa A.-C., Silva S.-L., Palma-Carlos M.-L., Palma-Carlos A.-G. - Hemoglobinopathies and asthma. *Asthma* 2001;2, suppl. 1, 156.
8. Efremov G.-D., Hui Sman T.-H. - La diagnosi di laboratorio delle emoglobinopatie. *Ematologia* 1974;3:132-181.
9. Embury S.-H. - Sickle cell anemia and associated hemoglobinopathies, pg 893-905 in *Cecil Textbook of Medicine, Goldman L., Bennett J.-C., (eds) Saunders, Philadelphia 2000.*
10. Galacteros F., Beuzard Y. - Hemoglobinopathies, pg.269-300 in *Hématologie. Dreyfus, B (ed) Flammarion Médecine-Sciences, Paris 1984.*
11. Hill M.-R., Cookson WOCM. - A new variant of the Beta subunit of the high affinity receptor for Immunoglobulin E (FcE RIBE2379) Association with measures of atopy and bronchial hyper-responsiveness, pg 120-128 in *Hum Med Genetic* 1996;959-962.
12. Holgate S. - The genetic basis of allergic disease, pg 189-202 in *Allergy, Holgate S., Church MK, Lichtenstein, LM. (eds) Mosby, London 2001.*
13. Klee G.-G. - Role of morphology and erythrocytic indices in screening and diagnosis, pg 120-128 in *Hemoglobinopathies and thalassemia Fairbanks VF (ed) Thorn-Stratton, New York 1980.*
14. Lehman H., Hunstman R.-G. - *Man's haemoglobins*. North Holland, Amsterdam 1974.
15. Lehman FRS., Kynoch P.-A. - *Human haemoglobin variants*. North Holland Publishing Company Amsterdam 1976.

- 16 - Lleyssen MHJ., Goossens WFJM., Verwilghen R.-L. - Concentration of Piracetam in erythrocytes, pg 157 in Abstracts, International Society of Hematology, European and Africam Division, Sevenmth meeting, Barcelona, 1983.
17. Miale J.-B. - Laboratory Medicine, Hematology. C.V. Mosby, 1967 St Louis.
18. Nechelles T.-F., Allen D.-M., Finkel H.-E. - Clinical disorders of hemoglobin structure and synthesis. Appleton - Century Crofts, New York, 1969.
- 19 - Palma-Carlos A.-G., Carmo J.-A. - Piracetam in Drepanocytosis. Symposium Piracetam, Lisbon, 1981;18.
20. Palma-Carlos M.-L., Palma-Carlos A.-G. - Talassemias. Conceitos actuais. O Médico (Lisboa) 1982;105:480-490.
21. Palma-Carlos M.-L., Palma-Carlos A.-G. - Abnormal Hemoglobinopathies in Center and South Portugal, pg 156 Proceedings VII Meeting International of Hematology, Barcelona 1983.
22. Palma-Carlos M.-L., Ferreira A., Palma-Carlos A.-G. - Talassemia e reacções de hipersensibilidade, pg 74 in XIV International Meeting in Allergology and Immunology GAILL, Lisboa 1989.
23. Palma-Carlos A.-G., Ferreira A., Palma-Carlos M.-L. - Talassemia and allergic diseases. Abstract Book World Asthma Congress Interasma 1990 Maebashi, Japan.
24. Palma-Carlos A.-G., Branco Ferreira M., Pereira dos Santos M.-C., Palma-Carlos M.-L. - The nose and the Bronchi: A functional unit. Asthma 2001;2 Supp. 1:97-102.
25. Palma-Carlos M.-L., Costa A.-C., Palma-Carlos A.-G. - Hemoglobinopathies and asthma, pg 125 in XXIII EAACI Congress, Abstract Book, Amsterdam 2004.
26. Palma-Carlos M.-L., Costa A.-C., Palma-Carlos A.-G. - Hemoglobinopathies and asthma, pg 20 in Abstract Book, American College of Allergy, Asthma and Immunology Annual Scientific Meeting, Boston 2004.
27. Rodgers G.-P. - Hemoglobinopathies: The thalassemias, pg 884-893 in Cecil's Textbook of Medicine, Goldman L., Bennett JC (eds) Saunders, Philadelphia 2000.
28. Weatherall D.-J., Clegg J.-B. - The thalassaemia syndromes, 1972 Blackwell Scientific, Oxford.
29. Weatherall D.-J. - Abnormal Hemoglobins and thalassaemia, pg 43-68 in Recent Advances on Haematology, Hoffbrand AV., Brain MC., Hirsch J. (eds) Churchill Livingstone, London 1977.
30. Weatherall D. - The thalassemias, pg 581-615 in Hematology, Beutler E., Lichtman MA., Collier BS., Kipps T (eds) McGraw-Hill 1995, New York.
31. Weatherall D.-J. - Protein synthesis in the red cell: the thalassemias, pg 630-683 in Blood and its disorders, Hardisty RM, Weatherall DJ (eds) Blackwell Scientific Publications, Oxford 1974.
32. Wintrobe M.-M., Lee Gr., Boggs D.-R., Bithell T.-C., Athens J.-W., Foerster J. - Clinical Hematology. Lea and Febyer, Philadelphia 1974.

