ORIGINAL ARTICLE

The clinical relevance of an isolated increase in the number of circulating hyperchromic red blood cells

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Aims: To search for laboratory evidence of hereditary spherocytosis (HS) among apparently healthy children with the chance finding of an isolated increase in hyperchromic red cells (cells with intracellular haemoglobin concentration > 410 g/litre).

Methods: Blood and reticulocyte counts and Pink tests were performed on successive children found on routine counts to have > 4% hyperchromic red cells, and compared with age and mean cell haemoglobin concentration (MCHC) matched controls and children known to have HS.

Results: Thirty four patients with > 4% hyperchromic red cells had significantly higher absolute numbers of such cells ($p < 0.0001$) and higher reticulocyte counts ($p < 0.01$) than age matched controls, together with higher MCHC ($p < 0.0001$) and haemoglobin distribution width ($p < 0.0001$) values and lower mean cell volume ($p < 0.02$) values. Significant differences were also found among hyperchromic red blood cell, reticulocyte, and haemoglobin distribution width values when subjects were compared with MCHC matched controls. Pink test values were higher in children with increased hyperchromic red blood cells, but not significantly so. In patients with HS, most variables measured were significantly different both from those of children with > 4% hyperchromic cells and controls. Despite the differences found, few MCHC, HDW, reticulocyte, or Pink test values were outside of the normal limits, and only one child with increased hyperchromic cells had both a mild reticulocytosis and a slightly raised Pink test value.

Conclusions: Subjects with an isolated increase in hyperchromic red blood cells have a profile of red blood cell changes similar to that of patients with HS, but to a lesser degree. They may carry a recessive form of the disease but lack the laboratory features of clinically manifest HS.

As a result of advances in automated red blood cell analysis, an increase in the proportion of hyperchromic red blood cells (cells with haemoglobin concentration > 410 g/litre) has become a consistent finding in subjects with hereditary spherocytosis (HS). The finding is of diagnostic value and can be of use in assessing the severity of the disorder.^{1–3} Red blood cells in HS are normally shaped when young but become progressively thicker with age. Mean cell volume (MCV) decreases slightly and mean cell haemoglobin (MCHC) increases as they acquire the spherocytic and hyperchromic appearances typical of the disorder. Normal red blood cell morphology may be found transiently in HS when the red cell population is shifted to a younger age—for example, after a period of aplasia.⁴ A much smaller proportion of hyperchromic cells is found in normal subjects and probably represents the dense cells formed as a result of red blood cell aging.

"Red blood cells in hereditary spherocytosis are normally shaped when young but become progressively thicker with age"

We have noted increases in hyperchromic red cells in a small proportion of apparently healthy children in the UK, and carried out our present study to determine whether such children have other laboratory evidence of HS.

METHODS

Blood count data (Technicon H1; Bayer, Newbury, UK) were obtained from children whose samples gave a hyperchromia flag, indicating the presence of $\geq 4\%$ hyperchromic red blood cells. In addition, the following tests were performed on each sample: flow cytometric reticulocyte count (Facsan; Becton Dickinson, Oxford, UK), modified glycerol lysis Pink test,⁵

direct antihuman globulin test, and haemoglobin (Hb) electrophoresis on cellulose acetate membrane at pH 6.8. The Pink test is a modification of the acidified glycerol lysis test in which 10 μ l of blood is suspended in a buffered sodium chloride/glycerol reagent for 30 minutes, after which the degree of haemolysis is measured. Absolute numbers of hyperchromic red blood cells and reticulocytes were calculated from percentage values and the automated red blood cell count. The same investigations were carried out on two groups of controls, one matched for age (within six months for children > 2 years of age, within 3 months for those < 2 years) and the other for MCHC (within ± 2 g/litre). Subjects and controls were essentially healthy children attending hospital for minor illnesses or planned surgery and, apart from hyperchromia flags in the test population, all had normal blood counts. The same panel of tests was also performed on children known to have HS. Each child was tested on one occasion only.

All tests were performed on skin puncture blood samples: blood counts were performed within one hour of collection and Pink tests within three hours. The additional tests required < 0.2 ml of blood in total and no child had extra blood taken for the purpose of our study. Results were compared using the Wilcoxon signed rank test.

Laboratory records of subjects and controls were studied to determine the incidence of a hyperchromia flag in blood count

Abbreviations: Hb, haemoglobin; HDW, haemoglobin distribution width; HS, hereditary spherocytosis; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; RDW, red blood cell distribution width

Table 1 Mean values (SD) and significance of differences between variables in age matched subjects and controls, and patients with HS

Variable	Subjects (n=34)	Controls (n=34)	HS patients (n=14)	p Value (subjects/controls)	p Value (HS/controls)	p Value (subjects/HS patients)
Hb (g/l)	131 (12)	127 (7)	112 (14)	NS	<0.0001	<0.001
MCV (fl)	79.3 (2.0)	82.0 (5.8)	78.3 (3.5)	<0.02	<0.05	NS
MCH (pg)	27.9 (1.3)	27.8 (2.0)	28.5 (1.6)	NS	NS	NS
MCHC (g/l)	351 (1.0)	339 (0.9)	366 (1.6)	<0.0001	<0.001	<0.01
RDW (%)	12.7 (0.6)	13.0 (1.0)	18.4 (3.9)	NS	<0.001	<0.0001
HDW (g/l)	29.8 (1.8)	25.6 (2.7)	41.5 (7.2)	<0.0001	<0.0001	<0.0001
Hyperchromic RBC ($\times 10^9$ /l)	329 (245)	51.8 (47)	1193 (579)	<0.0001	<0.0001	<0.0001
Reticulocytes ($\times 10^9$ /l)	102.8 (44.8)	76.0 (32.6)	445.7 (131.2)	<0.01	<0.0001	<0.0001
Haemolysis (%)	18.4 (11.0)	14.3 (10.3)	81.9 (15.2)	NS	<0.0001	<0.0001

Hb, haemoglobin; HDW, haemoglobin distribution width; HS, hereditary spherocytosis; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; NS, not significant; RBC, red blood cells; RDW, red blood cell distribution width.

samples analysed before and after the one on which the above tests were performed.

RESULTS

Samples from 34 consecutive children with a hyperchromia flag and sufficient remaining sample to perform the full range of tests were studied over a period of 12 months, during 1996 and 1997. Their ages ranged from 1 to 14.5 years, with a median of 6. Thirty four age matched controls were also studied and controls matched for MCHC were found for the 21 subjects with MCHC values < 352 g/litre. We were unable to find MCHC matched controls with higher values than this because there was always an associated hyperchromia flag. Fourteen children with HS aged 0.5 to 11 years, median 6, were also studied. None had been splenectomised. All subjects, controls, and children with HS had a negative direct antihuman globulin test and a normal Hb electrophoresis. Proportions of hyperchromic red blood cells were 0.3–3.9% in controls, 4.0–13.0% in the subject group, and 4.0–53.3% in children with HS.

Subjects had significantly higher numbers of hyperchromic red blood cells and reticulocytes than age matched controls, together with significantly higher values for MCHC and haemoglobin distribution width (HDW; a measure of anisochromia) and lower MCV values (table 1). They also had higher Pink test values than controls, although the difference was not significant.

Significantly higher numbers of hyperchromic red blood cells and reticulocytes, and higher HDW values were also found among the 21 subjects matched for MCHC with a control group (table 2).

As expected, the HS group showed highly significant differences compared with both the subject and control groups for most variables tested (table 1).

Four subjects (11.8%) had evidence of increased red blood cell turnover, defined as a reticulocyte count greater than the mean + 2 SD value of the control group; one also had a raised Pink test value. Two other children had raised Pink test values alone.

Twenty four of the 34 subjects studied had at least one blood count performed on another occasion. Of the 14 who had counts within six months of the date of the study sample, eight gave a hyperchromia flag on at least one other occasion, whereas six did not.

Among the remaining 10, one gave a flag on only one of nine samples analysed over a four year period, whereas two others gave flags on each of three samples analysed over three years and each of five analysed over 2.5 years, respectively. No hyperchromia flags were found in the blood count records of children comprising the control groups.

DISCUSSION

Increased numbers of hyperchromic red blood cells are found in a variety of red blood cell disorders other than HS, including immune haemolytic states, in association with HbC,⁶ (interaction of this common variant with the inner aspect of the red cell membrane leads to cellular dehydration and increased MCHC values), and in disorders of red blood cell fragmentation. The children in our subject group were free of such disorders and a mild form of HS seemed a possible cause of their blood count findings, therefore prompting further investigation.

The observation of significantly higher MCHC values and hyperchromic red blood cell numbers by comparison with the control group was to be expected from the study design. However, taken together with the significantly higher reticulocyte numbers and HDW values, these findings produce a profile of changes very similar to that found in patients with HS, albeit to a lesser degree (table 1). For instance, the reticulocyte counts and HDW values of children with HS were consistently raised above normal limits and MCHC values frequently so,

Table 2 Mean values (SD) and significance of differences between variables in subjects and controls matched for MCHC

Variable	Subjects (n=21)	Controls (n=21)	p Value
Hb (g/l)	131 (10)	126 (2)	NS
MCV (fl)	80.0 (2.5)	80.4 (5.8)	NS
MCH (pg)	27.7 (0.9)	27.8 (1.9)	NS
RDW (%)	12.6 (0.6)	13.0 (0.9)	NS
HDW (g/l)	29.6 (2.1)	26.4 (2.0)	<0.01
Hyperchromic RBC ($\times 10^9$ /l)	249 (106)	99 (100)	<0.01
Reticulocytes ($\times 10^9$ /l)	122 (51)	74 (32)	<0.01
Haemolysis (%)	19.3 (10.3)	13.7 (9.2)	NS

Hb, haemoglobin; HDW, haemoglobin distribution width; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; NS, not significant; RBC, red blood cells; RDW, red cell distribution width.

whereas in the subject group most such values were within the ranges found in the control group. This was also the case with the Pink test, where we found, as have others,⁷ that values of normal subjects and patients with HS overlap at haemolysis values around 40–45%. There were three such borderline results among the subjects and one in both the control and HS groups. The red blood cell distribution width (RDW) is typically raised in HS, and together with a raised MCHC is highly predictive of this disorder.⁸ Our findings in patients with HS support this conclusion, although in our study group RDW values were almost identical to those of the controls (tables 1,2).

It might be argued that the higher reticulocyte counts of the subject group are a compensatory feature secondary to their higher MCHC values, based on the premise that higher MCHCs are a marker of less deformable red cells, which are likely to have a reduced life span. We found no evidence for this, because significantly higher numbers of hyperchromic red blood cells and reticulocytes and higher HDW values persisted in the 21 subjects with less high MCHC values for whom MCHC matched controls could be found (table 2).

The proportion of hyperchromic red blood cells in our subjects ranged from 4.0% to 13.0%, a very similar range to that reported by Pilar Ricard and Gilsanz³ in patients with mild and moderate forms of HS. Whereas all their subjects also had increased reticulocyte numbers and positive osmotic fragility tests, only three of our subjects had a reticulocytosis, two had a borderline positive Pink test, and one had both findings. Although some subjects with very mild HS may give negative findings with the osmotic fragility group of tests,⁹ only one child, a 14.5 year old boy with a reticulocyte count of 169×10^9 /litre and a Pink test value of 41.6%, might be considered to have a mild form of the disorder.

“Our findings confirm the earlier reports based on osmotic fragility tests that about 1% of North European subjects have laboratory findings indicative of a continuum between normal subjects and those with clinically manifest hereditary spherocytosis”

The laboratory records of the subject group were studied to assess the likelihood that an increase in hyperchromic red blood cells was a transient phenomenon. Eight of 14 subjects gave at least one additional hyperchromia flag in blood counts analysed within six months of the date of the study sample. A further two subjects showed the continued presence of the abnormality over periods of 2.5 and three years, respectively. These findings indicate that in many children the abnormality is persistent, a conclusion supported by the associated higher reticulocyte counts and HDW values. Failure to demonstrate a small increase in hyperchromic cells consistently may be caused by mild physiological fluctuations within an essentially stable red blood cell population, sufficient to activate the analyser flag on some occasions but not on others.

Take home messages

- Subjects with an isolated increase in hyperchromic red blood cells have a profile of red blood cell changes similar to that seen in patients with hereditary spherocytosis (HS), including significantly higher numbers of hyperchromic red blood cells and reticulocytes, and significantly higher values for mean cell haemoglobin concentration and haemoglobin distribution width, and lower mean cell volume values, although these were seen to a lesser degree than is present in HS
- About 1% of North European subjects have laboratory findings indicative of a continuum between normal subjects and those with clinically manifest HS
- These individuals may carry a recessive form of the disease but lack the laboratory features of clinically manifest HS

Earlier searches for evidence of mild HS among 1008 Norwegian and 1464 German blood donors using the osmotic fragility and acidified glycerol lysis tests, respectively,^{10,11} produced positive findings in about 1% of those tested. Some of these subjects also had a mild reticulocytosis. We estimate the incidence of an increase in hyperchromic red blood cells in our population also to be around 1%, giving an incidence of an abnormal Pink test of about 0.1%, based on our finding of a borderline positive result in three of the 34 children studied. The reason for this approximate tenfold difference is unclear, although the number of children we tested is too small for meaningful comparison.

Our findings, using sensitive methods of red blood cell analysis and precise automated reticulocyte counts, confirm the earlier reports based on osmotic fragility tests that about 1% of North European subjects have laboratory findings indicative of a continuum between normal subjects and those with clinically manifest HS. Carrier status for a recessive form of HS might be the molecular basis for the clinically unimportant red blood cell changes in these individuals.

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REFERENCES

- 1 Pati, AR, Patton, WN, Harris, RI. The use of the Technicon H1 in the diagnosis of hereditary spherocytosis. *Clin Lab Haematol* 1989;11:27–30.
- 2 Ialongo P, Vignetti M, Cigliano G, et al. Flow cytometric measurement (H-1 Technicon) of microcytic and hyperchromic red cell populations in pediatric patients affected by hereditary spherocytosis (HS). *Haematologica* 1989;74:547–53.

- 3 **Pilar Ricard M**, Gilsanz F. Assessment of the severity of hereditary spherocytosis using routine haematological data obtained with dual angle laser scattering cytometry. *Clin Lab Haematol* 1996;**18**:75–8.
- 4 **Korones D**, Pearson HA. Normal erythrocyte osmotic fragility in hereditary spherocytosis. *J Pediatr* 1989;**114**:264–6.
- 5 **Vettore L**, Zanella A, Molaro GL, *et al*. A new test for the laboratory diagnosis of spherocytosis. *Acta Haematol* 1984;**72**:258–63.
- 6 **Ballas S**, Kocher W. Erythrocytes in HbSC disease are microcytic and hyperchromic. *Am J Hematol* 1988;**28**:37–9.
- 7 **Bucx MJL**, Breed WPM, Hoffman JJML. Comparison of acidified glycerol lysis test, Pink test and osmotic fragility test in hereditary spherocytosis: effect of incubation. *Eur J Haematol* 1988;**40**:227–31.
- 8 **Michaels LA**, Cohen AR, Zhao H, *et al*. Screening for hereditary spherocytosis by use of automated erythrocyte indexes. *J Pediatr* 1997;**130**:957–60.
- 9 **Dacie JV**. *The haemolytic anaemias*, Vol 1, 3rd ed. London: Churchill Livingstone, 1985.
- 10 **Godal HC**, Heisto H. High prevalence of increased osmotic fragility of red blood cells among Norwegian blood donors. *Scand J Haematol* 1981;**27**:30–4.
- 11 **Eber SW**, Pekrun A, Neufeldt A, *et al*. Prevalence of increased osmotic fragility of erythrocytes in German blood donors: screening using a modified glycerol lysis test. *Ann Haematol* 1992;**64**:88–92.

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