Comparison of haematological indices between women of four ethnic groups and the effect of oral contraceptives

IF GODSLAND, MARY SEED, RUTH SIMPSON, G BROOM, * V WYNN

From the Alexander Simpson Laboratory for Metabolic Research and the *Department of Haematology, St Mary’s Hospital Medical School, London W2

SUMMARY
Six-hundred-and-eighty-two women volunteers from four ethnic groups—Black, Indian, Oriental and White took part in a study to assess differences in haematological indices between the groups. This study was part of a broad investigation into the metabolic effects of oral contraceptives. The effect of the oral contraceptive (OC) on haematological indices was analysed but not found to be significant. The haemoglobin concentrations were lower than normal laboratory reference values for White subjects in 12.5% of Indian, 16.5% of Black and 4.3% of Oriental control groups respectively. All the White control group were within the normal range. These findings were considered in relation to age, weight, pregnancies, smoking, alcohol consumption, nutritional status, and disorders of haemoglobin synthesis.

A deficient iron intake accounted for the high incidence of low haemoglobin in the Indian group in whom low transferrin saturation and serum ferritin were observed.

With the possible exception of inherited disorders of haemoglobin synthesis, none of the factors we considered could account for the low haemoglobins found in the Black group. Separate reference values for haematological indices in different ethnic populations are considered.

In connection with an investigation into the metabolic effects of oral contraceptives in women of different ethnic groups, we became aware of a high proportion of low haemoglobin concentrations in our Black and Indian populations. If these results are taken as diagnostic for anaemia in Blacks and Indians, then we are confronted with a considerable health problem. However, it may be that the levels we observed are appropriate for healthy Black and Indian individuals.

The majority of laboratories in this country base their normal ranges on a predominantly White population. In investigating individuals from other ethnic groups such as the Black, Indian and Oriental populations now present in any large city in the UK, use of criteria based on White populations for assessing normality may be misleading.

Published studies of haematological differences between ethnic groups living in the same community are scarce. The most exhaustive studies have been between American Blacks and Whites. Analysis of data from two major nutritional surveys showed a mean haemoglobin (Hb) level in Blacks 1 g/dl less than in Whites. This difference was independent of age, economic level, nutritional status, and geographical area and has been confirmed in subsequent studies.

The possible contribution to these findings of inherited defects in Hb structure or deficiencies in Hb synthesis does not appear to have been extensively explored. A defective Hb characteristically associated with Black populations is HbS, which is present as a trait in 15% of Blacks but does not have any significant effect on haematological indices. However, non-fatal inherited diseases of Hb synthesis, classified under the thalassaemia syndromes, may be present in one-third of the Black populations and may significantly affect haematological indices.

Comparative studies between Indians and Whites are scarce. A high incidence of anaemia is believed to occur in Indian women as immigrants to the United Kingdom and the few studies that have been carried out suggest a nutritional origin for this anaemia. Surveys both on the Indian sub-continent and in the UK, suggest a significant incidence of thalassaemia among Indian populations, and this could
Comparison of haematological indices and the effect of oral contraceptives

Our study also included a group of Oriental subjects. Available evidence suggests no significant differences in the haematological indices between Orientals and Whites, although inherited disorders of globin chain synthesis do appear to be widespread.

The data we have analysed were derived from an investigation into the effects of the OC in women of different ethnic groups. The OC has been shown to change significantly parameters likely to affect haematological indices, but studies demonstrating this have only involved White women. Therefore, in assessing the effects of the oral contraceptive in other ethnic groups, an evaluation of haematological differences is essential, and a comparison of haematological indices in OC users and non-users in each ethnic group is included in the following study.

We have considered observed haematological differences between different ethnic groups with regard to possible contributing or causative factors. Age, weight, pregnancies, smoking, alcohol intake, incidence of infection or infestation, disorders of haemoglobin synthesis, iron status and general nutrition and dietary history were analysed for each ethnic group. With regard to iron status, serum ferritin, iron and total iron binding capacity (TIBC) were measured on a sample from each group. Total protein, albumin, serum calcium, phosphate and alkaline phosphatase, as well as dietary information, are considered with reference to general nutrition.

Subjects and methods

The majority of women in this study were volunteers responding to a printed circular. Women were grouped as Black, Indian, Oriental and White. To be included in a group, each subject had to have at least three grandparents belonging to that group. In the Black group, 50% were from the West Indies, 30% were from Africa and 20% were born in the UK of West Indian parents. In the Indian group, 35% were from India and Sri Lanka, 7% from Pakistan and Bangladesh, 42% from East Africa and 11% from the West Indies. Orientals came from the Far East and South East Asia and Whites were predominantly from the UK. Women were further divided into control groups if they had not taken the oral contraceptive for at least three months prior to the study, and OC groups if they had been taking the OC for three months or more.

Subjects attended a day investigation unit, where a medical and dietary history was taken. Blood was taken between 0900 and 0930 h, after a 12 h fast. To standardise the effect of posture on plasma volume dependent variables, subjects were semirecumbent for 30 min prior to blood sampling. Blood was obtained without stasis, from an in-dwelling venous cannula. Full blood counts were performed on a Coulter Counter, Model S, maintained within quality control limits using Coulter reference control 4C and an in-house QC preparation. The following indices were considered: MCV, RBC, PCV, Hb, MCH, MCHC and white cell count (WBC). Erythrocyte sedimentation rate (ESR) was measured (Westergren) and Blacks were screened for sickle cell trait by haemoglobin electrophoresis. An SMA screen was carried out for each subject, and parameters considered in this study are serum total protein, albumin, calcium, phosphate and alkaline phosphatase, each by standard Technicon methods. Serum ferritin was measured by a specific radioimmunoassay (Beckton & Dickinson, Product No 241130). Serum iron was measured by bathophenanthroline without deproteinisation (Boehringer, Mannheim, Product No 124222) and TIBC by magnesium carbonate precipitation (Boehringer, Mannheim, Product No 125806). Transferrin saturation was calculated by the serum iron expressed as a percentage of the TIBC.

Women were excluded from this study if they were already under treatment for any previously diagnosed disease. Women with ages outside the reproductive age range of 16-45 yr were also excluded. To assess differences in weight between different ethnic groups, weights were expressed as a percentage of each individual's ideal body weight. Percentage ideal body weight (% IBW) was taken as an expression of the height, weight, age relationship in each individual. Those with % IBW of greater than 150% were excluded from the study. Subjects with MCV less than 74 fl and RBC greater than 5·0 &times; 10^{12}/l were considered likely to have beta-thalassaemia trait and haemoglobin electrophoresis was carried out in these cases. Those with abnormally high HbA2 levels were excluded from the study. The numbers excluded are presented in Table 4 (see later). Final numbers in each group were: Black control 123, Black OC 142; Indian control 109, Indian OC 79; Oriental control 117, Oriental OC 59; White control 69, White OC 84.

Statistical analysis

Analysis of the distribution of the data showed variations in the form of the frequency distributions for each parameter between different ethnic groups. Therefore, in testing for significant differences, non-parametric techniques were used throughout. The Mann-Whitney U test was applied in testing for significant differences in a parameter between any two groups. In comparison between the four different ethnic groups a Kruskall-Wallis one way analysis of
Table 1  Medians and ranges (in parentheses) for haematological indices, age and percentage ideal body weight and pregnancies per person for control and OC groups in each ethnic group

<table>
<thead>
<tr>
<th></th>
<th>Blacks Controls</th>
<th>Blacks OC</th>
<th>Indians Controls</th>
<th>Indians OC</th>
<th>Orientals Controls</th>
<th>Orientals OC</th>
<th>Whites Controls</th>
<th>Whites OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of cases</td>
<td>123</td>
<td>142</td>
<td>109</td>
<td>79</td>
<td>117</td>
<td>59</td>
<td>69</td>
<td>84</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12-46</td>
<td>12-52</td>
<td>(8-9-14-5)</td>
<td>(10-2-14-2)</td>
<td>12-36</td>
<td>12-58</td>
<td>12-50</td>
<td>12-78</td>
</tr>
<tr>
<td>PCV</td>
<td>0-375</td>
<td>0-378</td>
<td>(0-300-0-433)</td>
<td>(0-296-0-434)</td>
<td>0-369</td>
<td>0-376</td>
<td>0-373</td>
<td>0-377</td>
</tr>
<tr>
<td>WBC</td>
<td>28-74</td>
<td>(65-100)</td>
<td>(3-35-7-67)</td>
<td>(3-32-3-34)</td>
<td>4-320</td>
<td>4-390</td>
<td>4-242</td>
<td>4-190</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>86-3</td>
<td>86-9</td>
<td>(69-99)</td>
<td>(69-97)</td>
<td>88-7</td>
<td>89-3</td>
<td>89-1</td>
<td>89-6</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>28-74</td>
<td>28-80</td>
<td>(20-8-32-7)</td>
<td>(20-2-32-4)</td>
<td>29-61</td>
<td>30-12</td>
<td>30-14</td>
<td>30-37</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33-06</td>
<td>33-01</td>
<td>(29-9-35-7)</td>
<td>(29-8-35-8)</td>
<td>33-33</td>
<td>33-50</td>
<td>33-68</td>
<td>33-58</td>
</tr>
<tr>
<td>WBC (× 10^3/L)</td>
<td>5-18</td>
<td>5-38</td>
<td>(3-9-3-9)</td>
<td>(3-9-3-9)</td>
<td>5-63</td>
<td>6-25</td>
<td>6-18</td>
<td>6-46</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>10-5</td>
<td>10-4</td>
<td>(2-9-5-14-5)</td>
<td>(2-9-5-14-5)</td>
<td>12-1</td>
<td>11-9</td>
<td>13-5</td>
<td>9-7</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>26-0</td>
<td>24-9</td>
<td>(17-45)</td>
<td>(17-42)</td>
<td>26-3</td>
<td>25-9</td>
<td>26-8</td>
<td>27-9</td>
</tr>
<tr>
<td>% IBW</td>
<td>105-3</td>
<td>104-9</td>
<td>(75-149)</td>
<td>(82-146)</td>
<td>89-4</td>
<td>90-3</td>
<td>102-3</td>
<td>102-5</td>
</tr>
<tr>
<td>No of</td>
<td>0-5</td>
<td>1-0</td>
<td>(0-8)</td>
<td>(0-14)</td>
<td>0-1</td>
<td>0-3</td>
<td>0-3</td>
<td>0-2</td>
</tr>
<tr>
<td>pregnancies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Significant differences between control and OC groups shown as: *p < 0.05; **p < 0.01; NS = not significant.

variance was applied and a significant result followed by tests between each pair of races.

Results

Within the range of median ages encountered in this study, a woman is considered to be anaemic if a haemoglobin less than or equal to 12.0 g/dl is found. The standardisation of posture used in this study would be expected to result in a 7% lowering of mean haemoglobin concentrations. We have confirmed this under the conditions of the present study. Blood for full blood counts was taken from 22 volunteers immediately on arrival and subsequently after 30 min in a semirecumbent position. A reduction of 7.8% was found in each of the plasma volume dependent variables: Hb RBC and PCV. Therefore, in this study we have considered a woman to be anaemic with a haemoglobin of less than or equal to 11.2 g/dl.

Since half of our subjects were OC users, it was necessary to determine whether the OC was having an effect on the haematological parameters under investigation. Medians, total ranges and numbers for each index and age, % IBW and average number of pregnancies per woman in control and OC groups are presented in Table 1. Also included is the significance of the difference between control OC groups for each parameter.

Numbers and percentages of women with low Hb in each control and OC group are presented in Table 2. Incidence of low values in the control group as compared with the OC is 3% more for the Blacks, 6.4% more for the Indians, and 2.6% more for the Orientals. In the White groups, only one anaemic individual was found and she was in the OC group.

In considering haematological differences between the different ethnic groups it is immediately apparent

<table>
<thead>
<tr>
<th>Blacks</th>
<th>Indians</th>
<th>Orientals</th>
<th>Whites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>15</td>
<td>12-2%</td>
<td>16-5%</td>
</tr>
<tr>
<td>OC</td>
<td>13</td>
<td>8</td>
<td>9-2%</td>
</tr>
</tbody>
</table>

Table 2  Numbers and percentages of individuals in control and OC groups in each ethnic group with Hb ≤ 11.2 g/dl
from Table 2 that low Hb concentrations are 7–18 times more prevalent among the Blacks and Indians than among Orientals and Whites. This difference is further emphasised when the haematological indices are compared between the four ethnic control groups from Table 1. Significance of the differences between the different ethnic groups are presented in Table 3. Blacks, Indians and Orientals all have significantly lower Hb concentrations than Whites.

In Table 4, the percentage of smokers, alcohol users, and vegetarians in each ethnic group is presented; also included are the percentages of those with confirmed beta-thalassaemia trait and sickle cell trait. Compared with the White group, the incidence of smoking in the other three groups was substantially reduced. Three quarters of Blacks and Whites took alcohol regularly. Vegetarianism was only a significant factor among the Indians of whom almost a quarter took no meat. Medians, total ranges, and numbers of cases considered in assessing the serum indicators of nutrition as shown in Table 5, and significant differences between the different groups are given in Table 6. The lowest serum ferritin, iron and transferrin saturation, and highest TIBC were observed in the Indian group.

The percentage of individuals in each group with low serum ferritin, iron and transferrin saturation are also presented in Table 5. Serum ferritin was considered to be abnormal at a value of ≤120 ng/l,18 serum iron at ≤7 μmol/l19 and transferrin saturation at ≤15%.20 The Indian groups contained substantially more individuals in these categories than any of the other groups.
Table 6  
Significances between ethnic groups nutritional indicators

<table>
<thead>
<tr>
<th></th>
<th>Overall Kruskall-Wallis test</th>
<th>Black v Indian</th>
<th>Black v Oriental</th>
<th>Black v White</th>
<th>Indian v Oriental</th>
<th>Indian v White</th>
<th>Oriental v White</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>NS</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Globulin</td>
<td>***</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phosphate</td>
<td>NS</td>
<td>*</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>NS</td>
<td>*</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ferritin</td>
<td>NS</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Iron</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TIBC</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* p < 0·05.
** p < 0·01.
*** p < 0·001.
NS = not significant.
Arrows indicate direction of the difference—for example, Indian v Oriental transferrin saturation ** = Indian median transferrin saturation is lower than Oriental median transferrin saturation.

Discussion

Some differences in haematological indices might be expected in groups of control subjects and OC users on the basis of reports of raised serum iron and TIBC, reduced menstrual blood loss; folate deficiency and reduced erythropoiesis associated with OC use.

Incidence of folate deficiency, as indicated by an MCV greater than 99 fl, was no higher in our OC groups than in our control groups. In our Black, Indian and Oriental groups, higher Hb and MCV levels were found in OC users, as seen in the medians for each group. The analysis of the numbers of individuals with low Hb from control and OC groups shown in Table 2 supported this. These results suggest that the effects of the OC might be beneficial in raising haematological indices. However, this effect was only statistically significant between the Hb levels in Oriental control and OC groups. The lack of a significant effect of OC use is particularly striking in the Black and Indian groups; although the OC reduces menstrual loss, there was no significant change in the already low Hb and MCV levels in these groups. Our findings agree with a study by Prema6 carried out in India in which the haematological effects of the OC and intrauterine device (IUD) were compared for patients attending a family planning clinic. No difference was found in either mean Hb or MCV levels between OC and IUD users.

The White controls had significantly higher Hb levels than the OC group, the only parameter associated with this difference was the number of pregnancies, but no correlation was found between this and Hb or MCV levels.

In the comparison of haematological data between the different ethnic groups, the most striking finding was the low Hb and MCV found in the Black and Indian groups. However, reference values derived from White populations may be inappropriate for our Black and Indian populations, where reference values may be lower as appears to be the case for American Blacks. This will only be so if the observed levels cannot be accounted for by any factor contributing to their incidence in one ethnic group rather than another. Such factors are age, weight, pregnancies, smoking, alcohol intake, nutritional status and inherited disorders of haemoglobin synthesis.

The effect of age on MCV is clearly demonstrated in men22 and may be so for women on OC.23 A weak positive correlation between age and MCV was found in our Black control and Mongolian control and OC groups, but there were no significant correlations in any other groups. There were no significant differences between the age distributions of the different ethnic groups.

The possible contribution of body mass to haematological differences between the races was difficult to assess. Percentage IBW was analysed to give an indication of the relation between weight, height and age. The Blacks in our study had the highest body mass for their age and the Orientals the lowest. Regression analysis showed that any effect of % IBW on Hb and MCV was negligible.

Women in each ethnic group had a significantly different number of pregnancies from those in each of the other groups. The number of pregnancies for women in this study ranged from 0–14, but possible effects on haematological indices associated with this parameter were discounted by the complete lack of correlation found between number of pregnancies and Hb and MCV.

There was a marked difference in smoking habits between women of different ethnic groups. Smoking
has been shown to raise both Hb and MCV\textsuperscript{23} and could thus account for the comparatively high levels of both these indices found in our White group. We therefore divided those women for whom information on smoking habits had been obtained, into smoking and non-smoking groups. Both median Hb and MCV levels were consistently higher in the smokers of each ethnic group. Comparisons between the non-smokers, Hb and MCV levels for each ethnic group resulted in there no longer being a significant difference between the Orientals and Whites. Incidence of smoking failed to account for the differences observed between the Whites, and Black and Indian groups.

Regular alcohol intake occurred in all groups. Liver function tests showed no evidence of alcohol abuse in any of our subjects. However, even moderate alcohol consumption has been known to raise the MCV.\textsuperscript{22} Three quarters of both Blacks and Whites in our study took alcohol, yet the Black median MCV was low relative to that of the Whites. The small effect of alcohol on the MCV that has been demonstrated would not account for any ethnic differences observed.

Chronic infection is known to be associated with anaemia and intestinal infestation has been shown to be an aggravating factor in the high incidence of anaemia found in the Indian subcontinent.\textsuperscript{24} These conditions would be expected to be accompanied by an increased incidence of eosinophilia, raised WBC and ESR which were not found. The Black WBC was significantly lower than in the other three groups in agreement with other studies.\textsuperscript{25}

Disorders of haemoglobin synthesis vary in their distribution among different ethnic groups. Beta-thalassaemia has the greatest effect on haematological indices and subjects with this trait were excluded from this study. The alpha-thalassaemias are less severe in their haematological effects, but can still significantly affect haematological indices.\textsuperscript{9} The double gene deletion, alpha-thalassaemia 1, has been reported in 5-6% of Black Americans,\textsuperscript{7} whereas single gene deletion alpha-thalassaemia 2, may appear in up to 27-5%,\textsuperscript{*} thus the thalassaemias could contribute substantially to the haematological differences between Black and White populations. However surveys in Black Americans did not correlate the incidence of either alpha 1 or 2 thalassaemia with haematological indices. Alpha-thalassaemia in Indians appears to have a genetic basis similar to that found in Blacks\textsuperscript{29} and incidences of 1-4% are reported.\textsuperscript{26} Among Orientals, studies in Thailand have demonstrated frequencies for alpha-thalassaemia 1 of up to 12-2% and for alpha-thalassaemia 2 of up to 17-4%.\textsuperscript{27} The alpha-thalassaemias also appear to be widespread in other parts of South East Asia and China. As alpha-thalassaemia alters haematological indices, we would expect a proportion of our subjects to have thalassaemic indices not associated with raised HbA\textsubscript{1C}, and three such cases did appear in our Black control group compared with only one Indian and one Oriental.

Analysis of dietary information showed that red meat was the principle source of protein in the diets of Blacks and Orientals. This was especially true of the Blacks whose nutritional status was reflected in their % IBW. In contrast, 22% of our Indians were vegetarians. Vegetarianism is not necessarily associated with anaemia, except in the inadequately nourished, and we did not find a greater number of anaemic individuals among our Indian vegetarians although they did show a lower median, Hb and MCV.

The analysis of indices of iron status provides strong evidence for a high incidence of iron deficiency in our Indian group. Nutritional iron deficiency is the cause of 90% of anaemia on the Indian subcontinent,\textsuperscript{24} and this is also associated with folate deficiency in 30–50% of subjects. A high incidence of nutritional anaemia is generally believed to extend to Indians as immigrants to the United Kingdom.\textsuperscript{10 11} Our Indian group showed lowest median ferritin, iron and transferrin saturation, and highest TIBC, and also the greatest proportion of individuals with low ferritin, iron and transferrin saturation levels. The White group gave median ferritin and iron levels lower than those of the Black and Oriental groups, although it showed the lowest proportion of individuals with ferritin levels of less than 12.0 ng/l. The low haemoglobin concentrations found in the Black population were not reflected in the indicators of iron status used in this study, and the relatively high median ferritin concentrations in Black and Oriental populations, thus appear to reflect a greater number of individuals with comparatively high ferritin concentrations in these groups, rather than iron deficiency in the White groups, in which there were no individuals with Hb less than 11.2 g/dl.

Our analysis of the iron status of our Indian population provides strong evidence for a nutritional basis for the low haemoglobin levels observed in this group. Reduced absorption or low intake of iron, or both, would be factors involved in this. Calcium, phosphate, and alkaline phosphatase levels did not indicate any interference with iron absorption by dietary chelating agents such as phytates. If low iron intake were the primary cause of anaemia in the Indian group, then iron supplementation would be expected to have a beneficial effect on haematological indices. This was found to be the case in a subsequent study in which 11 Indians were given oral iron over a period of two months. Serum iron,
TIBC, Hb, and MCV were measured before and after iron supplementation. Although median levels of these parameters before treatment did not fall outside the normal range, there was nevertheless a highly significant rise in both serum iron and transferrin saturation and significant increases in Hb and MCV after treatment.

The theory of reference values requires that reference individuals in a population should be comparable in a number of defined variables. It would appear from this study, that our group of Indian subjects were not comparable in iron and nutritional status with our White reference population. Diagnosis of anaemia in an Indian population can be effectively based on White reference values, though unequivocal diagnosis must await a reference value derived from an Indian group with adequate iron nutrition.

Our study indicates that reference values based upon White populations are applicable to the Orientals in our community, in whom adequate nutrition, iron status and iron stores, as estimated by ferritin levels, were associated with an incidence of low Hb concentrations similar to that in the normal White female population.

The low Hb concentration in our Black population is evidently not primarily due to nutritional factors, as indicated by dietary history and iron status. The possibility remains that there may be a prevalent but not easily detectable genetic impairment of haemoglobin synthesis in our Black population. However, a clear understanding of the contribution that the alpha-thalassaemias could make to these low Hb concentrations must await a thorough survey based on haemoglobin chain synthesis studies.

The haematological differences we have observed between Black and White populations agree with the findings of a number of workers in the USA where separate reference values are used. These differences are currently under discussion. On the strength of our findings, these may be equally applicable to the Black population in the UK.

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References


Requests for reprints to: Mr IF Godsland, Alexander Simpson Laboratory for Metabolic Research, St Mary's Hospital Medical School, London W2, England.