MEASUREMENTS OF IRON STATUS AND SURVIVAL IN AFRICAN IRON OVERLOAD

A Patrick MacPhail, Eberhard M Mandishona, Peter D Bloom, Alan C Paterson, Tracey A Rouault, Victor R Gordeuk

Introduction. Dietary iron overload is common in southern Africa and there is a misconception that the condition is benign. Early descriptions of the condition relied on autopsy studies, and the use of indirect measurements of iron status to diagnose this form of iron overload has not been clarified.

Methods. The study involved 22 black subjects found to have iron overload on liver biopsy. Fourteen subjects presented to hospital with liver disease and were found to have iron overload on percutaneous liver biopsy. Eight subjects, drawn from a family study, underwent liver biopsy because of elevated serum ferritin concentrations suggestive of iron overload. Indirect measurements of iron status (transferrin saturation, serum ferritin) were performed on all subjects. Histological iron grade and hepatic iron concentration were used as direct measures of iron status.

Results. There were no significant differences in either direct or indirect measurements of iron status between the two groups. In 75% of these subjects the hepatic iron concentration was greater than 350 $\mu g/g$ dry weight, an extreme elevation associated with a high risk of fibrosis and cirrhosis. Serum ferritin was elevated in all subjects and the transferrin saturation was greater than 60% in 93% of the subjects. Hepatomegaly was present in 20 of the 22 cases and there was only a moderate derangement in liver enzymes except for a tenfold increase in the median gamma-glutamyl transpeptidase concentration. There was a strong correlation between serum ferritin and hepatic iron concentrations ($r = 0.71$, $P = 0.006$). After a median follow-up of 19 months, 6 (26%) of the subjects had died. The risk of mortality correlated significantly with both the hepatic iron concentration and the serum ferritin concentration.

Conclusions. Indirect measurements of iron status (serum ferritin concentration and transferrin saturation) are useful in the diagnosis of African dietary iron overload. When dietary iron overload becomes symptomatic it has a high mortality. Measures to prevent and treat this condition are needed.

S Afr Med J 1999; **89**: 966-972.

Department of Medicine, University of the Witwatersrand, Johannesburg
A Patrick MacPhail, BSc, MB BCh, PhD, FCP (SA), FRCP
Eberhard M Mandishona, MB ChB
Peter D Bloom, MD

Department of Anatomical Pathology, University of the Witwatersrand and South African Institute for Medical Research, Johannesburg
Alan C Paterson, MB BCh, PhD

Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, Bethesda, Maryland, USA
Tracey A Rouault, MD

Department of Medicine, The George Washington University Medical Center, Washington, DC, USA
Victor R Gordeuk, MD

African iron overload, first described in 1929,[1] has not been well characterised in living subjects. Early studies were based on autopsy findings and documented an association with chronic liver disease,[2-4] specifically portal fibrosis and cirrhosis. Later studies also described associations with diabetes mellitus,[5] osteoporosis and scurvy,[6] and cardiomyopathy.[7,8] Hepatic iron deposition in African iron overload occurs in parenchymal cells, Kupffer cells and portal macrophages.[9] This is in contrast to Caucasian hereditary haemochromatosis, in which condition iron deposition is mainly parenchymal.[10] In both conditions widespread deposition of iron may occur in other organs.[3]

The development of African iron overload has been associated with the consumption of traditional beer rich in iron leached from the steel containers used in brewing.[11-13] Recent studies in southern Africa provide strong evidence that there is a genetic predisposition to African iron overload.[14,15] Understanding of the condition has been compromised by the widely held belief, based on the prominence of macrophage iron, that African iron overload is a benign condition and that alcohol plays the major role in causing hepatic disease.

Despite the fact that African iron overload has been recognised for more than 65 years and is still common in rural Africa in the 1990s,[16] it is not clear which indirect measurements of iron status correlate with chemical hepatic iron concentration, the gold standard in characterising iron overload.[17] The purpose of this study was to bring together the clinical and pathological features of iron overload seen in subjects who were recruited as part of a larger family study aimed at establishing a genetic factor in African iron overload,[15] and to examine the hypothesis that mortality correlates with the severity of iron overload.

MATERIALS AND METHODS

Study subjects

Twenty-two subjects aged 25 - 84 years with iron overload confirmed on liver biopsy, were studied between 1993 and 1996

Table I. Traditional beer consumption, hepatic histology and measurements of iron status in 22 subjects with African iron overload

Selection criteria	Subject No.	Age (yrs)	Sex	Lifetime beer consumption (l)	Hepatic iron: Hepatocytes	Hepatic iron: Kupffer cells	Hepatic iron: Macrophages	Hepatic histology: Fibrosis	Hepatic histology: Cirrhosis	Transferrin saturation (%)	Serum ferritin (μg/l)	Hepatic iron (μmol/g)	Hepatic iron index	Vitamin C (μg/10⁸ wbc)	Status
Liver	1	55	F	68 544	3	3	3	4	1	105	38 483	428	7.8	7.8	Dead
biopsy	2	56	M	31 616	3	3	3	1	0	81	17 991	557	9.9	4.6	Dead
	3	84	M	141 960	4	4	4	4	1	91	6 914	1 035	12.3	0.7	Dead
	4	84	M	12 090	3	3	3	4	1	99	6 716	627	7.5	9.9	Alive
	5	71	M	186 150	3	4	3	1	1	98	5 074	694	9.8	2.5	Dead
	6	55	M	16 992	4	3	3	1	0	101	4 968	.		21.4	Alive
	7	80	M	10 176	3	3	4	1	1	96	4 307	.	.	3.7	Alive
	8	65	M	28 440	4	4	4	1	0	86	4 016	173	2.7	4.0	Alive
	9	67	M	31 584	3	4	3	4	1	96	3 091	612	9.1	12.4	Dead
	10	69	M	6 528	3	3	3	1	1	104	2 938	577	8.4	14.1	Alive
	11	51	M	14 808	3	3	3	1	0	93	2 459	361	7.1	16.8	Alive
	12	56	M	7 488	3	3	4	1	0	94	2 062	.	.	6.2	Alive
	13	65	F	30 912	3	3	3	3	0	95	1 900	468	7.2	13.3	Alive
	14	69	M	6 048	3	3	3	3	0	92	1 331	.	.	9.4	Alive
Raised	15	65	M	41 472	3	3	4	2	1	92	13 108	725	11.2	6.4	Dead
ferritin	16	70	F	67 200	3	3	3	3	0	93	3 655	438	6.3	9.0	Alive
serum	17	69	M	67 160	3	3	3	3	0	87	3 009	558	8.1	12.1	Alive
	18	75	M	147 840	3	4	3	4	1	103	2 718	275	3.7	7.0	Alive
	19	70	F	17 280	2	2	1	1	0	35	2 162	82	1.2	13.6	Alive
	20	54	M	84 000	3	1	2	2	0	31	1 180	217	4.0	18.0	Alive
	21	25	F	72	2	2	1	1	0	108	1 026	.	.	44.7	Alive
	22	60	M	1 584	2	1	1	1	0	67	773	.	.	25.4	Alive

as part of a larger genetic study on iron overload based at Shongwe Hospital, Mpumalanga.[15] Fifteen of these patients were identified as index cases when they presented to hospital with hepatomegaly and other features of liver diseases (abdominal pain, ascites, haematemesis, hepatic encephalopathy and/or peripheral oedema), and the diagnosis of iron overload was made on liver biopsy. A further 8 subjects, from 2 of the above patients' families, underwent diagnostic liver biopsy when they were found to have elevated serum ferritin concentrations suggestive of iron overload. A medical history was taken and physical examination was performed on each subject. The volume of traditional beer consumed was estimated by direct questioning of each subject. A typical indigenous clay pot or *udzivo*, employed as a measure when selling beer, was used to assess the amount imbibed each day. The total lifetime beer consumption (in litres) was calculated from the daily or weekly consumption and the number of years spent drinking. Fasting morning blood samples were collected by venipuncture on 2 consecutive days.

Collection and analysis of traditional beer samples

Forty-eight samples of traditional beer that were ready for consumption were collected from homes in the Shongwe community and frozen immediately at -70°C. The iron concentrations in the beer supernatants were measured after acid digestion using bathophenthroline disulphonate at 535 nm absorbance. The alcohol concentration in the beer was determined by ultraviolet spectroscopy.[18]

Analysis of liver biopsy specimens

A direct estimate of iron status was obtained by grading the liver biopsy histologically and by measuring the chemical iron concentration. Hepatocyte iron was graded histologically on sections stained with Perl's reagent using the method of Scheuer and colleagues.[19] Kupffer cell iron and portal tract macrophage iron were each assessed separately. Iron grades were assigned according to the consensus of four observers (ACP, VRG, APM, EMM) observing the specimen simultaneously and blinded to the results of the indirect measurements of iron status. Fibrosis and cirrhosis were assessed on the basis of haematoxylin and eosin, Masson's and reticulin stains. The hepatic non-haem iron concentration was measured on dewaxed samples prepared from the histology blocks,[20] and the hepatic iron index (μmol iron/g liver tissue (dry weight)/age)[21] was calculated.

Indirect measures of iron status

Serum iron and total iron binding capacity were measured using methods recommended by the International Committee for Standardisation in Haematology.[22,23] The transferrin saturation was calculated by dividing the serum iron by the total iron binding capacity and multiplying by 100, with a maximum of 100%. The unsaturated iron binding capacity was the difference between the total iron binding capacity and the serum iron, with a minimum of 0. The serum ferritin concentration was measured by enzyme-linked immunosorbent assay (ELISA).[24] The ratio of serum ferritin to aspartate aminotransferase (AST) measured on day 1 was calculated because this ratio reflects hepatic iron stores in the setting of acute alcohol consumption, shortly after stopping and after up to 3 weeks' abstention from alcohol.[25,26] The mean of two results measured on blood samples drawn on consecutive days was used in the indirect measurements of iron status.

Haematological and biochemical tests

The full blood count was performed using an automated cell counter (Sysmex K 1 000, CA Milsch, Kobe, Japan). Serum concentrations of lactate dehydrogenase (LDH), AST, alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γGT), bilirubin, glucose and sodium were measured using an automated clinical chemistry analyser. Erythrocyte sedimentation rate (ESR) was determined using the Westergren method. C-reactive protein was measured by immunoturbidimetric assay (Boehringer Mannheim, Germany). The reticulocyte count was determined microscopically with peripheral blood smears stained with supra vital stain. Hepatitis B viral markers (hepatitis B surface antigen (HBsAg), surface antibody (anti-HBs), core antibody (anti-HBcore)) were measured by radio-immunoassay (RIA) (Abbot Diagnostics, Chicago, USA), and antibody to hepatitis C virus (anti-HC) was measured by ELISA (Murex Diagnostics, Temple Hill, Kent, England). White blood cell ascorbic acid concentration was determined according to the method of Denson and Bowers.[27]

Statistical methods

Continuous variables were compared with the Mann-Whitney *U*-test and categorical variables were compared with the Fisher exact or the Pearson chi-square test. Linear regression was used to examine relationships between variables. Hepatic iron stores do not correlate well with serum ferritin concentration at levels greater than 4 000 μg/l.[28] This study includes 3 subjects (see Table I) in whom serum ferritin was greater than 10 000 μg/l and in whom the serum ferritin values are randomly scattered in the data. As regression analysis of the data identifies these values as outliers (studentised residual absolute values greater than 2), these values were not included in the comparison of direct and indirect measures of iron status. The Spearman correlation coefficient was used to analyse indirect measures of iron status according to the histological grades of hepatic iron. Cox proportional hazards models were used to analyse the influence of hepatic iron and serum ferritin concentrations on time to death.

RESULTS

Iron-related measurements

Table I lists the iron-related features and liver biopsy findings in each of the subjects. The iron-related features are summarised in

Table II. Exposure to traditional beer and measurements of iron status in 22 subjects with African iron overload (continuous variables are medians and ranges)

Characteristic	Result	Normal range
Age (years)	66 (25 - 84)	
Female subjects (*N* (%))	5 (23)	
Estimated traditional beer consumption		
Lifetime total (l)	29 676 (72 - 186 150)	
Years of exposure	40 (2 - 65)	
Amount consumed per day (l)	3 (0.4 - 14)	
Measures of iron status		
Direct measurements		
Hepatocellular iron grade (median (range))	3 (2 - 4)	0 - 1
Hepatic iron concentration (μmol/g dry weight)*	512 (82 - 1 035)	< 30
Hepatic iron index (μmol/g dry weight/years)	7.6 (1.2 - 12.3)	≤ 1.0
Indirect measurements		
Transferrin saturation (%)	93 (31 - 100)	20 - 45
Unsaturated iron binding capacity (μg/dl)	14 (0 - 197)	100 - 300
Total iron binding capacity (μg/dl)	212 (113 - 304)	250 - 400
Serum ferritin (μg/l)	3 050 (773 - 38 483)	20 - 400
Ferritin/AST ratio (μg/U)	56 (5 - 193)	< 15
Vitamin C (μg/10^8 white blood cells)	9.7 (0.7 - 44.7)	≥ 20

*N = 16.

Table II. There were no significant differences in the age, sex distribution, lifetime traditional beer consumption, chemical hepatic iron concentration or biochemical measurements of iron status between those subjects selected on the basis of iron overload on liver biopsy and those selected on the basis of raised serum ferritin who subsequently had a liver biopsy. Significant differences were encountered in the hepatic iron grading on histological examination (see 'Liver histology and iron measurements' below).

Exposure to dietary iron

The exposure to traditional beer varied from a calculated lifetime consumption of 72 litres to over 150 000 litres and there was also a wide variation in the amount of traditional beer claimed to be consumed per session, ranging from 1 to 14 litres per session. An inverse correlation was found between the estimated amount of traditional beer consumed per day and the serum sodium concentration, as a marker of haemodilution, lending some credence to these claims ($r = 0.59$, $P = 0.003$). The mean (SD) alcohol concentration of 48 samples of home-brewed beer collected from the subjects' households was 3.2% (0.4%) with a mean iron content of 46 (17) mg/l. In terms of exposure to alcohol and iron this translates into a median of 96 g of alcohol and 138 mg of iron per drinking day. Sixty per cent of the subjects admitted to drinking commercial 'Western-type' alcohol occasionally, mostly in the form of beer, while the consumption of spirits was very low.

Liver histology and iron measurements

The histological grade of hepatocyte (parenchymal) iron was above the normal range in all study subjects: grade 2 in 3, grade 3 in 16 and grade 4 in 3 (Table I). The distribution of iron in the liver varied from heavy iron deposits (4) in portal macrophages with moderate parenchymal iron to massive parenchymal iron (4) and little portal macrophage iron (1). In all instances, Kupffer cell iron was present, with a gradation of mild (1) to heavy (4). The group selected on liver biopsy had significantly higher iron grades in hepatocytes, Kupffer cells and macrophages than the group selected on the basis of raised serum ferritin concentration ($P = 0.013$, 0.025 and 0.025, respectively). Fibrosis was present in all cases and cirrhosis was detected in 9 (41%) of the needle biopsies, but this figure may be an underestimate because the detection of fibrosis and cirrhosis is unreliable in needle biopsies. There was no significant difference in the degree of fibrosis or the prevalence of cirrhosis between the two groups. While 2 of the subjects (1 in each group) had mild steatosis, no other features of liver damage attributable to alcohol were seen. In 16 subjects there was sufficient biopsy material available for the direct chemical measurement of hepatic iron. The median hepatic iron concentration was 512 μmol/g dry weight (range 82 - 1 035) and in 12 cases (75%) concentrations were greater than 360 μmol/g dry weight, the level associated with high risk for the development of cirrhosis in both African iron overload[2] and Caucasian hereditary haemochromatosis.[21] In all but 1 subject, the hepatic iron index was greater than 1.9, the level used to distinguish between iron-loading due to alcohol and that caused by hereditary haemochromatosis.[21, 29, 30] No statistically significant difference in iron concentration or hepatic iron index was detected between the two groups.

Indirect measurements of iron status

The transferrin saturation was greater than 60% in 93% of the subjects and was mirrored by the low median unsaturated binding capacity. In three-quarters of the subjects the transferrin saturation was greater than 90%. Seventy-seven per cent of the subjects had a total iron binding capacity lower than normal, a reflection of low plasma transferrin concentration characteristic of iron overload. The serum ferritin was markedly elevated in all subjects. In 3 of the subjects, 1 of whom had an hepatocellular carcinoma, the serum ferritin concentration was greater than 10 000 μg/l. The ferritin/AST ratio was greater than 15 in all but 1 case, suggesting that alcohol alone was not the cause of the high ferritin values. Nineteen of the 22 cases (86%) had white cell vitamin C concentrations below the normal range.

Correlation between direct and indirect measurements of iron status

Serum ferritin concentration correlated significantly with the hepatic iron concentration ($r = 0.71$, $P = 0.006$) (Fig. 1). Similarly, serum ferritin correlated significantly with the histological grading of hepatocellular (rho = 0.48, $P = 0.03$), Kupffer cell (rho = 0.70, $P = 0.007$) and portal macrophage iron (rho = 0.54, $P = 0.03$). The relationship of hepatic iron concentration with transferrin saturation ($r = 0.51$) and unsaturated iron binding capacity ($r = 0.55$) were also significant ($P < 0.04$).

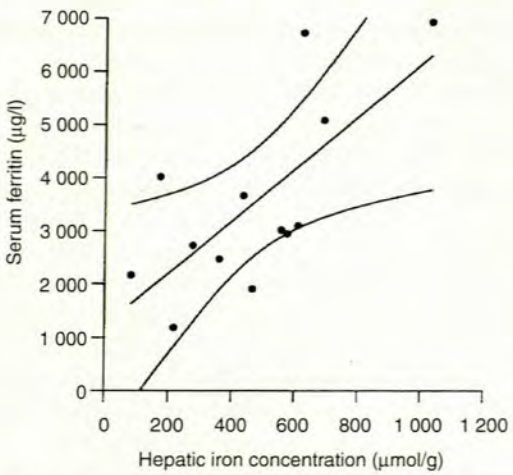

Fig. 1. Plot of serum ferritin and hepatic iron concentration in 13 subjects with African iron overload. Three subjects with serum ferritin concentrations greater than 10 000 µg/l were excluded (see methods).

Clinical features and other laboratory measurements

Clinical features and other laboratory measurements are shown in Fig. 2 and in Tables I - III. Apart from the erythrocyte sedimentation rate (see below), there were no significant differences in these features between those subjects selected on the basis of iron overload on liver biopsy and those selected on the basis of raised serum ferritin. The most common clinical finding was hepatomegaly, which was present in 91% of the subjects. A quarter of these subjects had ascites. More than half of the subjects complained of joint pain. Only 1 subject had clinical evidence of scurvy despite low white blood cell levels of vitamin C (< 20 µg/10^8 white cells) in 19 subjects (86%). One-fifth had a history of treated pulmonary tuberculosis. Two subjects had a fasting blood glucose greater than 6.6 mmol/l and 3 had clinical evidence of cardiomegaly. The median haematological measurements were within the normal range. The erythrocyte sedimentation rate was elevated above normal in most of the subjects, with the group selected on the basis of iron overload on liver biopsy having a significantly higher mean erythrocyte sedimentation rate (61 (29) and 32 (20) mm in 1 hour, respectively, $P = 0.01$). Abnormalities in liver function tests were common, particularly the γGT, which was above

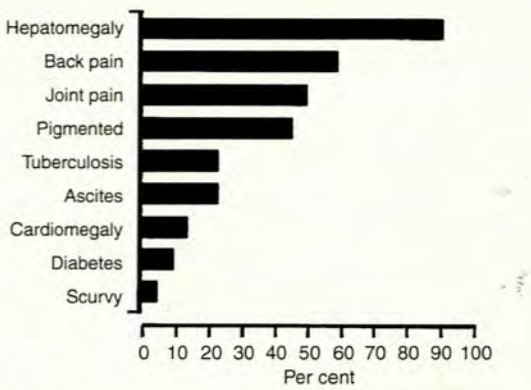

Fig. 2. Clinical features seen in 22 subjects with iron overload.

Table III. Haematology and biochemistry in 22 subjects with African iron overload (continuous variables are medians and ranges)

Haemoglobin (g/dl)	13.3 (10.5 - 15.3)	12.0 - 18.0*
Reticulocytes (%)	0.8 (0.3 - 1.9)	< 2.0
Erythrocyte sedimentation rate (mm/h)	49 (4 - 114)	< 20
C-reactive protein (mg/l mean)	2 (0 - 174)	≤ 2
Glucose (mmol/l)	4.8 (2.3 - 14.0)	< 6.6
Sodium (mEq/l)	138 (121 - 146)	135 - 145
Bilirubin (mmol/l)	8 (2 - 147)	< 25
Aspartate aminotransferase (U/l)	63 (14 - 405)	< 35
Alanine aminotransferase (U/l)	41 (7 - 172)	< 35
Gamma-glutamyl transpeptidase (U/l)	234 (55 - 1 836)	< 35
Viral hepatitis markers		
Hepatitis A antibody (*N* (%) positive)	22 (100.0)	negative
Hepatitis B core antibody (*N* (%) positive)	14 (64)	negative
Hepatitis B surface antibody (*N* (%) positive)	13 (59)	negative
Hepatitis B surface antigen (*N* (%) positive)	1 (5)	negative
Hepatitis C antibody (*N* (%) positive)	0 (0)	negative

*Values in the left-hand column are normal values.

normal in all cases. Hepatitis virus markers showed that all but 1 of the subjects had been exposed to the hepatitis A virus, while none had evidence of hepatitis C infection. Eighteen (78%) had evidence of past exposure to hepatitis B virus, but only 1 (5%) was positive for hepatitis B surface antigen. No subject showed evidence of chronic viral hepatitis on liver histology.

Mortality

After a median follow-up of 19 months (range 1 - 45 months), 6 subjects (26%) from this selected group with ages ranging from 5 to 84 years had died. Kaplan-Meier estimation shows that in this selected group of subjects with severe iron overload the probability of death was 38% at 22 months (Fig. 3). All but 1 of the deaths occurred in the group selected on the basis of iron overload on liver biopsy. One died from hepatocellular carcinoma while the remainder died from hepatic failure. In 3 who had ascites, liver failure followed haematemesis, possibly from bleeding oesophageal varices. In a Cox proportional hazards analysis, both hepatic iron concentration ($P = 0.023$) and serum ferritin ($P = 0.01$) were significantly related to mortality. The relative risk of death for each 100 μmol/g dry weight increase in hepatic iron concentration was 1.6 (95% CI, 1.1 - 2.3) and for each order of magnitude increase in serum ferritin (e.g. 300 - 3 000 μg/l) the relative risk was 9.6 (95% CI, 1.7 - 55.0).

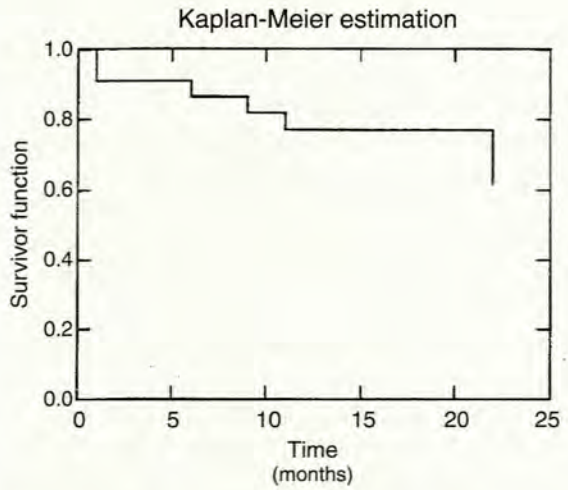

Fig. 3. Estimated survival (Kaplan-Meier) of 22 subjects with African iron overload selected from a larger genetic study.[15]

Discussion

Our study of 22 subjects selected from families taking part in a larger genetic study shows that the degree of iron overload in African blacks reaches massive proportions and confirms the impression of previous workers that this condition is distinct from alcoholic liver disease.[9] In 75% of the subjects the hepatic iron concentration was above 360 μmol/g dry weight, a level far greater than occurs in alcoholics,[21,31] and is associated with a high risk of hepatic damage.[2] Furthermore, while 2 subjects had minor histological features of alcoholic liver disease, this was against a background of severe iron overload. Finally, the hepatic iron index was above 1.7 in all save 1 subject, while among alcoholics it is never greater than 1.7.[21,29,30] Our results confirm that African blacks with iron overload often achieve hepatic iron levels that are similar to those found in homozygous hereditary haemochromatosis, but the distribution is notably different, with parenchymal, Kupffer and portal macrophage iron deposition being prominent in African iron overload, whereas iron deposition is mainly parenchymal in homozygous hereditary haemochromatosis.[10]

In the present study there was no difference in the direct or indirect measurements of iron status between the two groups of subjects that were selected on the basis of hepatic iron, a direct measurement, or elevated serum ferritin concentration, an indirect measurement. The results indicate that serum ferritin concentration is a good indirect marker of iron status in African dietary iron overload (Fig. 1). As in other disorders of iron metabolism, no single indirect test is diagnostic of African iron overload. The results also indicate that African iron overload can be strongly considered in a patient with the constellation of hepatomegaly, elevated serum ferritin, a transferrin saturation greater than 60% and exposure to home-brewed traditional beer. Such individuals are likely to have chemical evidence of tissue vitamin C deficiency and mild to moderate liver dysfunction. γGT concentrations may be markedly elevated. The cause of the marked elevation in γGT is unclear, except that it is probably not a marker of alcohol intake in this setting, but may reflect the presence of large amounts of iron in macrophage cells, the primary source of hepatic γGT.[32]

While Bothwell and colleagues[9] have clearly shown that African iron overload causes liver disease, especially portal fibrosis and cirrhosis, there is a widely held belief that African iron overload is a benign condition. Past studies have linked African iron overload to a variety of conditions, including carcinoma of the oesophagus and cardiomyopathy.[7,8] Recent evidence suggests that iron overload in Africans may be associated with an increased risk of death due to hepatocellular carcinoma and tuberculosis.[33] In addition, African iron overload has recently been shown to be a significant risk factor for the development of hepatocellular carcinoma.[34] While only a population-based study will provide a reliable estimate of mortality, the present study suggests that the mortality in subjects with established iron overload is high and that the risk of death is directly related to the degree of iron overload. Higher mean ESR in subjects presenting to hospital suggests the presence of inflammation in these individuals.

This study has shown that African iron overload is a serious condition with a significant mortality that can be readily diagnosed by clinical examination and simple indirect blood tests. Few surveys on the prevalence of African iron overload in sub-Saharan Africa have been conducted, but estimates range from 5% in the general population[35] to 21% in rural traditional beer drinkers.[13] Wide variation in prevalence was found among men from different regions of southern Africa.[36] These figures suggest that iron overload is still a major health problem in

rural Africa and that means of preventing and treating this condition need urgent attention.

This work was supported by grants from the US Office of Minority Health to the Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development (USA), the South African Medical Research Council and the University of the Witwatersrand.

References

1. Strachan AS. Haemosiderosis and haemochromatosis in South African natives with a comment on the aetiology of haemochromatosis. MD thesis, University of Glasgow, 1929.
2. Bothwell TH, Bradlow BA. Siderosis in the Bantu. A combined histopathological and chemical study. *Archives of Pathology and Laboratory Medicine* 1960; **70:** 279-292.
3. Isaacson C, Seftel HC, Keeley KJ, Bothwell TH. Siderosis in the Bantu: The relationship between iron overload and cirrhosis. *J Lab Clin Med* 1961; **58:** 845-853.
4. Bothwell TH, Isaacson C. Siderosis in the Bantu, a comparison of incidence in males and females. *BMJ* 1962; **1:** 1522-1524.
5. Seftel HC, Keeley KJ, Isaacson C, Bothwell TH. Siderosis in the Bantu: the clinical incidence of haemochromatosis in diabetic subjects. *J Lab Clin Med* 1961; **58:** 837-844.
6. Lynch SR, Berlowitz I, Seftel HC, *et al*. Osteoporosis in Johannesburg Bantu males, its relationship to siderosis and ascorbic acid deficiency. *Am J Clin Nutr* 1967; **20:** 799-807.
7. MacPhail AP, Simon MO, Torrance JD, Charlton RW, Bothwell TH, Isaacson C. Changing patterns of dietary iron overload in black South Africans. *Am J Clin Nutr* 1979; **32:** 1272-1278.
8. Swift PJ. Dietary iron overload as a cause of idiopathic cardiomyopathy in South Africa. *S Afr Med J* 1996; **86:** C17-C21.
9. Bothwell TH, Abrahams C, Bradlow PA, Charlton RW. Idiopathic and Bantu siderosis. *Archives of Pathology and Laboratory Medicine* 1965; **79:** 163-168.
10. Brink B, Disler P, Lynch S, Jacobs P, Charlton R, Bothwell T. Patterns of iron storage in dietary iron overload and idiopathic haemochromatosis. *J Lab Clin Med* 1976; **88:** 725-731.
11. Bothwell TH, Seftel H, Jacobs P, Torrance JD, Baumslag N. Iron overload in Bantu subjects. Studies on the availability of iron in Bantu beer. *Am J Clin Nutr* 1964; **14:** 47-51.
12. Buchanan WM. Bantu siderosis with a special reference to Rhodesian Africans. *University College of Rhodesia, Faculty of Medicine, Research Lecture Series* 1967; **1:** 5-30.
13. Gordeuk VR, Boyd RD, Brittenham GM. Dietary iron overload persists in rural sub-Saharan Africa. *Lancet* 1986; **1:** 1310-1313.
14. Gordeuk VR, Mukiibi J, Hasstedt SJ, *et al*. Iron overload in Africa: interaction between a gene and dietary iron content. *N Engl J Med* 1992; **326:** 95-100.
15. Moyo VM, Mandishona E, Hasstedt SJ, *et al*. Evidence of genetic transmission in African iron overload. *Blood* 1998; **91:**1076-1082.
16. Gordeuk VR. Hereditary and nutritional iron overload. *Balliere's Clin Haematol* 1992; **5:** 169-186.
17. Finch CA. The detection of iron overload. *N Engl J Med* 1982; **307:** 1702-1703.
18. Boehringer Mannheim. *Methods of Biochemical Analysis and Food Analysis*. Mannheim, Germany: Boehringer Mannheim GmbH, 1989: 40-42.
19. Scheuer PJ, Williams R, Muir AR. Hepatic pathology in relatives of patients with haemochromatosis. *J Path Bact* 1962; **84:** 53-64.
20. Torrance JD, Bothwell TH. A simple technique for measuring storage iron concentrations in formalinised liver samples. *S Afr J Med Sci* 1968; **33:** 9-11.
21. Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 1986; **6:** 24-29.
22. International Committee for Standardisation in Haematology (Iron Panel). Recommendations for measurement of serum iron in human blood. *Br J Haematol* 1978; **38:** 291-294.
23. International Committee for Standardisation in Haematology (Iron Panel). The measurement of total and unsaturated iron-binding capacity in serum. *Br J Haematol* 1978; **38:** 281-290.
24. Conradie JD, Mbhele BEL. Quantitation of serum ferritin by an enzyme-linked immunosorbent assay (ELISA). *S Afr Med J* 1980; **57:** 282-287.
25. Prieto J, Barry M, Sherlock S. Serum ferritin in patients with iron overload and with acute and chronic liver disease. *Gastroenterology* 1975; **68:** 523-533.
26. Ford C, Wells FE, Rogers JN. Assessment of iron status in association with excess alcohol consumption. *Ann Clin Biochem* 1995; **32:** 527-531.
27. Denson KW, Bowers EF. The determination of ascorbic acid in white blood cells: a comparison of white blood cell ascorbic acid and phenolic acid excretion in elderly patients. *Clin Sci* 1961; **21:** 157-162.
28. Worwood SJ, Jacobs A, Mclaren C, Ricketts C, Economidou J. Binding of serum ferritin to concavalin A: Patients with homozygous ß thalassaemia and transfusional iron overload. *Br J Haematol* 1980; **46:** 409-416.
29. Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. *Hepatology* 1990; **12:** 20-25.
30. Sallie RW, Reed WD, Shilkin KB. Confirmation of the efficacy of hepatic tissue iron index in differentiating genetic haemochromatosis from alcoholic liver disease complicated by alcoholic haemosiderosis. *Gut* 1991; **32:** 207-210.
31. Chapman RW, Morgan MY, Laulicht M, *et al*. Hepatic iron stores and markers of iron overload in alcoholics and patients with idiopathic hemochromatosis. *Dig Dis Sci* 1982; **27:** 909-916.
32. Goldberg DM. Structural, functional, and clinical aspects of gamma-glutamyltransferase. CRC Critical Review *Clin Lab Sci* 1980; **12:** 1-58.
33. Mandishona E, MacPhail AP, Gordeuk VR, *et al*. Dietary iron overload as a risk factor for hepatocellular carcinoma in black Africans. *Hepatology* 1998; **27:** 1563-1567.
34. Gordeuk VR, McLaren CE, MacPhail AP, Deichsel G, Bothwell TH. Associations of iron overload in Africa with hepatocellular carcinoma and tuberculosis: Strachan's 1929 thesis revisited. *Blood* 1996; **87:** 3470-3476.
35. Nesamvuni AE. Iron status in relation to dietary iron intake in adult Blacks residing in the Cape Peninsula. M.Nutr. thesis, University of the North, 1995.
36. MacPhail AP, Derman DP, Bothwell TH, *et al*. Serum ferritin concentrations in black miners. *S Afr Med J* 1979; **55:** 758-760.

Accepted 4 Jan 1999.