

Elevated iron indices in patients with diabetes

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Abstract

Aims Excess iron has been implicated in the pathogenesis of diabetes and its complications. This study documents the assessment of plasma iron indices and the correlation between transferrin saturation with biochemical and clinical parameters in a cross-sectional survey of 820 patients with diabetes in long-term follow-up in a single clinic.

Methods Plasma iron indices, together with the biochemical and clinical profile of all patients, were recorded over a 2-year period. Predictors of the transferrin saturation were identified using multiple and logistic regression analysis.

Results Eighty per cent of patients had Type 2 diabetes. The prevalence of elevated transferrin saturation (> 35%) was 3–4-fold higher in patients with diabetes, compared with historical prevalence described in the general population. Independent associations with elevated transferrin saturation were male gender, low C-reactive protein, and increased fasting plasma glucose (all $P < 0.0001$). Patients with Type 1 diabetes were also more likely to have an elevated transferrin saturation [odds ratio 3.9 (95% CI 1.9–8.0), $P < 0.001$]. Patients with an elevated transferrin saturation were younger, but had a similar duration of diabetes, possibly suggesting an earlier age of onset. There was no correlation between the presence of diabetic complications and the presence of elevated iron indices.

Conclusions Elevated iron indices are more common in patients with diabetes. Excess iron may have a role in the development of diabetes and subsequently in glycaemic control. This should be balanced by the strong association between iron indices and anaemia in patients with diabetes.

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Keywords anaemia, cross-sectional study, diabetes mellitus, iron, transferrin saturation

Abbreviations CRP, C-reactive protein; EI, elevated iron indices; FPG, fasting plasma glucose; Hb, haemoglobin; NI, normal iron indices; TSAT, transferrin saturation

Introduction

Excess iron has been implicated in the pathogenesis of diabetes and its complications [1–6]. Free iron serves as a catalyst for lipid and protein oxidation and the formation of reactive

oxygen species. In addition, iron indices are correlated with obesity and insulin sensitivity [5]. These factors have led some to promote iron chelation as a possible adjunctive therapy in diabetes [1]. Despite these associations, no systematic survey has established the prevalence and predictors of elevated iron indices in diabetes. This study aimed to establish the prevalence rate of elevated iron indices in a cross-sectional survey of patients with diabetes and determine possible clinical and biochemical correlations.

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Patients and methods

Study design

This study was designed as a cross-sectional survey of patients with diabetes in long-term follow-up at the Austin and Repatriation Medical Centre, Melbourne, Australia. 'Long-term follow-up' was defined as patients having at least three estimations of albumin excretion rate (AER). This strategy effectively excluded recently diagnosed patients (< 1 year). A 2-year window was chosen to include the review pattern of all patients currently enrolled in this clinic (i.e. at least bi-annual follow-up). Using this criterion, 820 patients with diabetes were identified. Results were obtained with the informed consent of patients following approval by the Austin Clinical Research Ethics committee. Iron indices were compared with historical prevalence data available for age-strata in the Australian population [7].

Plasma and urine biochemistry

Standard indices were recorded from blood testing, including serum creatinine, urea, albumin, fasting plasma glucose, fasting lipid profile, full blood count, HbA_{1c}, and C-reactive protein (CRP). Urinary creatinine, urea, albumin and protein were obtained from a 24-h collection. In addition, iron storage indices including ferritin (immunoturbidimetric method, Hitachi 917 Automatic analyser), serum iron and unsaturated iron binding capacity (Ferrozine method, Hitachi 917 Automatic analyser) were assessed. Transferrin saturation (TSAT) was then calculated according to standard formulae. These methods were similar to those used in the Australian prevalence survey [7]. Glomerular filtration rate (GFR) was estimated using the MDRD-6 formula [8]. Clinical data including anthropomorphic measurements, age, race, gender, BMI, type and duration of diabetes, length of follow-up, and a clinical history detailing the presence or absence of specific diabetic complications and additional treatment modalities were obtained from patient records.

'Elevated iron indices (EI)' were defined as a TSAT > 35%, to facilitate direct comparison with available Australian prevalence data [7]. 'Normal' iron indices (NI) were arbitrary, defined as a TSAT between 20 and 35%. TSAT was used in preference to ferritin to determine elevated iron indices, as systemic inflammation in patients with diabetes makes the interpretation of iron stores from ferritin questionable. Nonetheless, the 90th centile level for men and women in the age-matched Australian population was used as a surrogate marker for 'elevated ferritin levels' in patients with diabetes.

Statistical methods

Although matched for the age and gender of our population, statistical assessment with historical Australian data detailing the prevalence of elevated TSAT levels is not appropriate as data were obtained separately. Nonetheless, general population data are provided for indirect comparison. Continuous data are expressed as mean \pm SEM. Differences in continuous variables were compared using Student's *t*-tests (two groups) or one-way ANOVA (three or more groups, where subgroups were compared

using Fisher's PLSD post-hoc test). Differences in categorical variables were compared using χ^2 analysis. Pearson correlation was used to analyse univariate associations between continuous variables. Multivariate analysis utilized multiple regression and analysis of covariance (ANCOVA) to model the independent predictors of iron indices. Logistic regression was used to estimate odds ratios for statistically independent risk factors for EI expressed as a binary outcome.

Results

Outpatient population

The study included 458 men (56%) and 362 women, followed up for a median of 4.8 years (range 1–28 years). The mean age was 62.2 \pm 0.5 years (range 17–88). The mean duration of diabetes was 16 years. The majority of participants (80%) had Type 2 diabetes. Twenty-seven per cent had microalbuminuria and 12% had macroalbuminuria. The mean estimated GFR was 73.5 \pm 1.3 ml/min/1.73 m², with 30% of both men and women having moderate renal impairment (GFR < 60 ml/min/1.73 m²). Forty per cent of patients in our survey had documented vascular disease (32% having a history of ischaemic heart disease, 10% cerebrovascular disease and 12% peripheral vascular disease). Half of the patients in this survey were obese (BMI > 30 kg/m²) which is approximately twice that seen in the general (age-gender-matched) Australian population [9]. The mean BP was 143 \pm 1 mmHg (systolic) and 80 \pm 0.6 mmHg (diastolic). The mean lipid levels were: total cholesterol 4.7 \pm 0.1 mmol/l, LDL cholesterol 2.8 \pm 0.1 mmol/l, triglycerides 1.7 \pm 0.1 mmol/l, HDL cholesterol 1.3 \pm 0.1 mmol/l. Sixty-eight per cent of patients were receiving anti-hypertensive therapy, 56% received lipid lowering therapy and 20% of patients with Type 2 diabetes also received insulin. Additional baseline characteristics of this population have been previously reported elsewhere [10].

Prevalence of abnormal iron indices

In patients with diabetes, 11% had EI (14% of men and 7% of women). This rate is approximately 3–4 times that seen in the age-matched Australian population. The prevalence of elevated ferritin levels was also increased in patients with diabetes. By comparison, the prevalence of low iron storage indices (TSAT < 16%) was comparable or less than that seen in the age-gender-matched adult Australian population (Table 1). However, the mean TSAT was higher in our cohort than seen in the general population and the mean serum iron levels in our cohort were slightly lower.

Predictors of elevated iron indices

Using multiple logistic regression analysis, EI was independently associated with male gender, low CRP, and a high fasting plasma glucose (FPG) (Table 2). In addition, patients with Type 1 diabetes were more likely to have EI compared with

Iron indices	Women (n = 362)	Men (n = 458)	P
TSAT (mean ± SEM)	21.2 ± 0.5	25.5 ± 0.4	< 0.0001
Ferritin (median, iqr)	82 [19–132]	152 [53–266]	< 0.0001
TSAT > 35%	7.0% (2.2%)	14% (3.6%)	< 0.001
Ferritin > 90th centile (for age-matched general population)	12% (10%)	15% (10%)	0.29
TSAT < 16%	28.9% (27.7%)	12.9% (19.6%)	< 0.0001
Ferritin < 12 µg/l	5.5% (4.6%)	0.9% (0.7%)	< 0.001
Iron deficiency ^a defined as TSAT < 16% + ferritin < 12 µg/l	4.3% (3.6%)	0.7% (0.4%)	< 0.001

Age-matched general Australian population (in brackets).

P-values refer to gender differences (*t*-test for TSAT, Mann–Whitney test for ferritin and chi square tests for prevalences).

^aIron deficiency definition proposed by Life Sciences Research Office and the US National Center for Health Statistics.

Table 1 Iron indices in patients with diabetes compared with the age-matched general population

Risk factor	χ^2	P	Odds ratio (95% CI)	Increased risk (%)
Type 1 diabetes	13.7	< 0.001	3.9 (1.9–8.0)	290 (90–700)
CRP < 3.0	11.7	< 0.001	3.4 (1.7–6.8)	240 (70–580)
FBG > 11.0	5.1	0.02	2.2 (1.1–4.5)	120 (10–350)
Sex: male	4.8	0.03	2.3 (1.1–4.8)	130 (10–380)

Table 2 Adjusted odds ratios for risk factors for elevated transferrin saturation (> 35%) in patients with diabetes

those with Type 2 diabetes (odds ratio = 3.9 (95% CI 1.9–8.0), $P < 0.001$). These associations were not eliminated when patients without complications were considered separately and remained equally predictive in patients with Type 1 or Type 2 diabetes. Despite urinary iron losses associated with proteinuria [11], AER was not correlated with TSAT levels. HbA_{1c} was also associated with iron indices ($P < 0.01$), but this reflected the correlation between FPG and HbA_{1c} ($r = 0.51$) and HbA_{1c} was not associated with EI after adjustment for FPG.

Patients with EI had significantly higher haemoglobin levels than patients with normal TSAT levels, with only 6% having anaemia, compared with 25% in the diabetic population as a whole [10]. GFR was not significantly different in patients with elevated iron indices, although hyperfiltration (GFR > 130 ml/min/1.7 m²) was slightly more common in this subgroup (EI; 3.8%, NI; 0.6%, $P = 0.03$).

Clinical associations

Although menstrual status was not formally assessed, approximately two-thirds of the women with diabetes were > 60 years and were therefore likely to be amenorrhoeic. Of women < 50 years (probable pre-menopausal), 4.3% had EI levels, compared with 5.3% of women aged > 60 years (probable post-menopausal, $P = 0.76$). Overall, patients with EI were on average younger than those with NI. Despite this, the duration of diabetes was similar, suggesting that patients with EI may have an

earlier age of onset of diabetes. However, in this cohort, interpreting the age of diagnosis data is questionable given that the correlation between age and age of diagnosis is very high (Type 1, $r = 0.63$; Type 2, $r = 0.73$) and often unreliable in patients with Type 2 diabetes. Patients with EI had less cardiovascular disease (CVD) ($P = 0.05$), coronary disease ($P < 0.04$) than patients with low TSAT levels (< 16%), reflecting the association between anaemia and vascular disease. However, the rate of CVD was not different to that seen in patients with NI. Fewer patients with EI were receiving an ACE inhibitor ($P < 0.04$), but achieved blood pressure control was not correlated (vs. systolic BP, $P = 0.25$). However, none of these clinical differences remained significant after adjustment for CRP and/or FPG. Notably, neither smoking history, type of anti-diabetic treatment, nor anthropomorphic measurements including BMI were significantly associated with EI within our population.

Discussion

This cross-sectional survey documents a high prevalence of EI in a large cohort of patients with diabetes. Eleven per cent of our cohort overall had EI, which is approximately 3–4 times greater than that previously described in the Australian general population [7]. These population prevalence data do not represent a true control group and interpretation of these results is therefore problematic. Although matched for age and gender,

a number of socio-economic, geographical and dietary differences between outpatients from a tertiary referral clinic and the general population may contribute to this excess. In addition, a number of clinical indices including BMI, GFR and CRP, known to be positive predictors of iron stores in elderly patients [12] could not be controlled for. Nonetheless, our findings are consistent with data from the NHANES III survey which demonstrated an increased prevalence of high iron indices in patients with newly diagnosed diabetes both in men (odds ratio 4.94) and women (odds ratio 3.61) [6]. It is possible that the excess of diabetic patients with EI reflects the implicated role of iron in the development of insulin resistance and diabetes [4]. Consistent with this hypothesis, patients with EI may have an earlier age of onset of diabetes than patients with normal or low iron indices.

Patients with the highest TSAT levels were men with Type 1 diabetes and poor glycaemic and lipid control. In addition, TSAT levels were also influenced by systemic inflammation. This association was not removed when patients without these complications were considered separately. Although women had significantly greater CRP levels than men ($P < 0.01$), this difference could be eliminated after adjusting for gender differences in TSAT, suggesting a possible link between iron and the basal inflammatory status. It should also be noted that this survey may be biased by the impaired survival of patients with elevated iron indices. However, in contrast to previous reports [13], there was no evidence that the rate of disease progression or prevalence of complications was significantly related to EI. In fact, the opposite could be suggested, as patients with low TSAT (and Hb) were more likely to have a history of cardiovascular, cerebrovascular or peripheral vascular disease than those with NI or EI. This is consistent with data from the RENAAL study showing impaired outcomes in patients with diabetes with declining Hb levels (for which the main determinant is TSAT [10]), even within the so-called 'normal' range [14].

We demonstrated that glycaemic control was also correlated with EI. While this association may be partially explained by poor nutrition or dietary over-vigilance, improvements in glycaemic control achieved by iron reduction supports a direct role for iron and strengthens the call for chelation in patients with EI [1]. It is also possible that the association with glycaemic control reflects the interaction between hyperinsulinaemia with iron indices [15,16]. This is further supported by evidence suggesting that hyperglycaemia precedes the elevation of iron indices in diabetes [6]. Against this, some studies suggest that glycated transferrin has reduced iron-binding capacity [17].

It remains unclear what should be done to manage the iron indices of patients with diabetes. The influence of iron on cardiovascular risk remains unresolved. In the presence of hyperglycaemia and inflammation, iron may contribute the development and progression of oxidative injury. Iron may also negatively impact on glycaemic control. However, iron indices are strongly correlated with Hb, which represents an important risk factor for morbidity and mortality in patients

with diabetes, particularly in patients with established cardiovascular disease [10,14]. Ideally, the clinical approach should be a careful balance between insufficient iron stores and excess iron. As an alternative strategy, the mobilization of accumulated tissue iron and the maintenance of erythropoiesis via supplementation with recombinant erythropoietin may have a role for selected patients.

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