The effect of iron and erythropoietin treatment on the HbA1c of patients with diabetes mellitus and chronic kidney disease.

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Objectives: To examine the effect of intravenous iron and erythropoietin stimulating agents (ESA) on the glycemic control and HbA1c of patients with diabetes mellitus (DM) and chronic kidney disease (CKD).

Research Design and Methods: This was a prospective study of patients with Type 2 DM and CKD stage IIIIB or IV undergoing intravenous iron (Group A) and/or ESA (Group B). Full blood profiles were determined over the study period. Glycemic control was monitored using HbA1c, seven point daily glucose 3 times weekly and continuous glucose monitoring (CGMS).

Results: There were 15 patients in both Group A and B. Mean HbA1c (95% confidence interval) values fell in both groups (7.40% (6.60, 8.19) to 6.96% (6.27, 7.25), p<0.01 with intravenous iron and 7.31% (6.42, 8.54) to 6.63% (6.03, 7.36), p=0.013 on erythropoietin). There was a no change in the mean blood glucose in Group A (9.55 (8.20, 10.90) vs. 9.71 (8.29, 11.13) mmol/L, p=0.07) and in Group B (8.72 (7.31, 10.12) vs. 8.78 (7.47, 9.99) mmol/L, p=0.61) over the study period. Hemoglobin and haematocrit values significantly increased following both treatments. There was no linear relationship found between the change in HbA1c values and the rise of hemoglobin following either treatment.

Conclusions: Both iron and ESA can cause a significant fall in HbA1c values without a change to glycemic control in patients with DM and CKD. At the present time, regular capillary glucose measurements and the concurrent use of CGMS remain the best alternative measurements of glycemic control in this patient group.
continuous glucose monitoring (CGMS) devices. Thus, any class effect that iron therapy and ESA may have on HbA1c values could in fact represent a parallel change to glycemic control along with the currently postulated physiological changes. Furthermore, the effect of the fall in HbA1c following these 2 therapies have not been well studied in patients not already on haemodialysis. This study therefore sought to establish how intravenous iron and ESA therapy influence HbA1c values in patients with Type 2 diabetes mellitus (T2DM) and chronic kidney disease not on haemodialysis. Robust monitoring of blood glucose was performed throughout the study period to determine if the anticipated fall in HbA1c was a true reflection of glycemic control.

**RESEARCH DESIGN AND METHODS**

This was a prospective study of patients with T2DM and CKD stage IIIIB or IV (estimated glomerular filtration rate MDRD 15-44 ml/min/1.73 m²) selected for treatment with intravenous iron and/or erythropoietin stimulating agents between January 2009 and December 2009 inclusive. All patients were attending a single renal service where the decision to commence iron and ESA therapy was made by the attending physician. The study consisted of 2 groups. The first group (A) were patients selected for iron therapy according to clinical need and the second group (B) of patients were those needing ESA treatment. Glycemic control in both patient groups was assessed in the month leading up to treatment and once again for a 4 week period 4 months after therapy. These assessments comprised the measurement of glycated haemoglobin (HbA1c), 7 point glucose day profiling (7PGM) 3 times weekly and continuous glucose monitoring (CGMS) for a minimum of 48 hours. A more detailed account of the study methodology and patients are described below.

**Patient selection and exclusion criteria:**

**Iron Therapy Group (A)—**All patients selected for iron therapy had either absolute or functional iron deficiency as evidenced by serum ferritin values < 200 µg/L. All patients had hemoglobin ≤10.5 g/dl. Patients in this group were not on previous or concurrent ESA therapy and were vitamin B12 and folate replete. Intravenous iron was given as a single dose in the form of low molecular weight iron dextran (Cosmofer) dependent on the patient’s body weight. This was delivered as an initial intravenous test infusion of 100mg of iron over one hour followed by the remaining dose over the next 2-4 hours.

**Erythropoietin stimulating agent (ESA) therapy Group (B)—**All patients receiving ESA therapy had hemoglobin ≤10.5 g/dl and were considered iron, vitamin B12 and folate replete prior to initiation. Patients were considered iron replete following a serum ferritin value > 200 µg/L or having received intravenous iron at least 6 weeks prior to ESA therapy. ESA treatment was given in the form of darbepoetin alpha at 750 µg/kg fortnightly and continued throughout the period of the study. The dose of ESA was titrated monthly to achieve a target hemoglobin 10.5 – 12 g/dl.

**Exclusion criterion:** Patients with known hemoglobinopathy, history of transfusion or bleeding with the last 6 months, previously treated with ESA, on renal replacement or with previous transplantation were excluded from the study.

**Sample analysis and monitoring of glycemic control:** Patients in groups A and B were provided with the *Abbott*...
**Freestyle Freedom Lite** glucose sensor (Abbott Diagnostics, Maidenhead, UK). Patients were requested to perform 7 point glucose monitoring (7PGM) 3 times weekly one month before commencement of treatment until the end of the study. 7PGM was defined as pre-meal, 90 min post meal and pre-bed capillary glucose measurements.

Continuous glucose monitoring (CGMS) was performed using the **Medtronic CGMS Ipro Continuous Glucose Recorder** (Medtronic Minimed, Northridge, US). Using this system, measurements of interstitial glucose levels were made 228 times over a 24 hour period. Calibration of CGMS readings were made based on patient 7PGM over the similar time period.

All patients had CGMS performed for 2-4 days. This was done prior to ESA and iron therapy and once again at the end of the study.

Results from the 7PGM and CGMS were downloaded from their respective meters for data analysis. Results from the CGMS included at least a successful 24 hour profile over the monitoring period with no gaps > 120 mins.

The management of diabetes control were left to the patients and their health care professional. Treatment for glycemic control was monitored throughout the study period.

Blood was drawn fasting from all patients for HbA1c and full blood profile. All HbA1c measurements were made using ion-exchange chromatography via the Menarini HA-8160 HbA1c analyser (A. Menarini, Berkshire, United Kingdom). It has been shown that there is no interference between carbamylated hemoglobin (present in uremia) and HbA1c using this analyser [12].

Patients in group A and B had samples taken at the one month before commencement of therapy and once again 4 months following treatment initiation.

**Data analysis.** All data was tabulated using Microsoft Excel and statistical analysis was made using SPSS 16.0 using paired t tests where appropriate. Mean blood glucose (MBG) pre and post treatment was calculated by taking the average of the daily mean glucose values where there were 3 more capillary glucose readings per day. As glucose values were measured more frequently over CGMS monitoring periods, the results were weighted to ensure each measurement was proportional to the inverse of the total number of measurements taken the same day similar to that done in the A1c-Derived Average Glucose (ADAG) Study [13].

**Power calculation:** Data from previous studies were used to calculate the statistical power required in the knowledge that iron has previously been shown to have a larger effect on HbA1c than ESA [6, 14]. Assuming the intrasubject variation of HbA1c is Gaussian [15], 9 patients were required to detect a 1.2% fall in HbA1c in group A and 13 patients to detect a 1.0% fall in group B with 80% power to an alpha of p<0.05 using nQuery (Statistical Solutions Ltd, Cork, Ireland).

Ethical approval was obtained from the Local ethics committee prior to the commencement of the study. (LREC number 08/H1304/114)

**RESULTS**

**Patient data. Intravenous iron therapy (Group A)—**Fifteen patients (9 M 6 F; all caucasian, median age 72 years (IQR 68-74), median albumin to creatinine ratio (ACR) 6.3 (IQR 4.3-76.3)) agreed to participated in this arm of the study. Six patients were diet controlled and 9
patients were insulin requiring. The mean±SD follow up period was 16.4±3.7 weeks.

**Erythropoietin Stimulating Agent Therapy (Group B)**—Fifteen patients (11 M 4 F; all caucasian, median age 70 years (IQR 62-75), median ACR 9.3 (IQR 6.0-93.4)) were recruited in this group. Four patients were diet controlled, 4 were on oral hypoglycemic agents and 7 were insulin requiring. The mean follow up time in this group was 17.3±3.3 weeks. No patient received additional oral or intravenous iron therapy over the period of the study following the initiation of ESA treatment.

**Glucose measurements and control.**
No new treatments affecting glycemic control (e.g. oral hypoglycemic agents, steroids, β blockers) were initiated or altered over the study period in all patients.

The CGMS and the 7PGM data included ~ 1300 and 250 measurements per subject, respectively, for a total of ~ 1500 glucose tests over the entire study period. Using the 7PGM results there are a mean 4.7 readings a day of which 31% seven point profiles were complete. The median days of CGMS were 6. The results of the CGMS were retrospectively calibrated with the 7PGM readings performed over the similar period.

MBG in both groups did not change over the study period. The results of these are summarised in Table 1 and 2.

**HbA1c values.** Despite a lack of change of glycemic control in the both groups, HbA1c concentrations fell significantly (p<0.001 and 0.013 respectively for Groups A and B). There was no linear relationship between the change in HbA1c and hemoglobin concentration values. (Group A, Pearson’s 2 tailed, $R^2=-0.329$, p=0.23, Group B, $R^2=-0.313$, p=0.25)

**Subgroup analysis of Group B.** In the group of patients receiving ESA therapy, there were 7 patients (5 M, 2F, median age 72 (IQR 62-79)) who received ESA therapy after iron treatment and 8 patients (6M,2F, median age 69 (IQR 61-74) who were received ESA only. All patients who also received iron were treated at least 6 weeks prior to ESA therapy initiation.

There appeared to be a non significant trend towards ESA leading to a further decrease in HbA1c following the initial fall due to iron (mean HbA1c 7.3% to 6.9%, p=0.36 following iron and 6.9% to 6.7%, p=0.13, following ESA). In contrast, the group of patients receiving ESA therapy without iron had a significant fall in HbA1c from 7.3% to 6.5%, p =0.02.

MBG did not change in either group (9.12 vs. 9.21 mmol/L, p=0.47, ESA and iron vs 8.21 vs 8.26 mmol/L, p=0.71, ESA only) and there was a concurrent rise to hemoglobin (9.6 to 11.76 g/dl, p<0.01 vs 9.4 to 11.3 g/dl, p<0.01) and hematocrit values (0.310 to 0.347, p<0.01 vs 0.331 to 0.384) following therapy.

**CONCLUSIONS**

Erythropoietin stimulating agents and intravenous iron are commonly used therapies in the management of anemia in patients with CKD. Patients with both DM and CKD have a higher prevalence of severe anemia as compared to patients with CKD alone [16-18]. Despite the increased usage of ESA agents, recent findings have shown that the correction of anaemia to levels of haemoglobin in excess of 12.5g/dl in patients with T2DM using this therapy has not led to an improvement in mortality but rather an increased risk of stroke. This needs to be interpreted carefully as the two groups received disproportionate amounts of IV iron. Indeed in the placebo group it was...
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noted that there was an increase in the haemoglobin levels with ESA agents. Hence best practice would suggest that correction of functional and absolute iron deficiency should be obtained prior to commencement of ESA [19].

This is the first study to robustly show that iron and erythropoietin stimulating agent treatment result in a fall in HbA1c which is independent of glycemic changes in patients with DM and CKD stage IIIb and IV.

Discordantly high HbA1c values as compared to glucose readings have been reported in previous studies and case reports on non DM patients with iron deficiency [10, 11, 20, 21] and in patients with T1DM in childhood and pregnancy [22]. The correction of the iron deficiency in all these patient groups has lead to a fall in HbA1c values in these patients though the monitoring of glycemic control of patients has not been as robust as compared to our study (using methods such as fasting plasma glucose or 2 pre meal readings a day).

Several studies have also shown a fall in HbA1c concentrations following ESA treatment in patients with DM undergoing haemodialysis [7, 8]. Other than a single case report [23], there was scarce data to support the class effect of this therapy on patients not on haemodialysis.

Nakao et al. [7] reported a fall in HbA1c in non DM patients with CKD on haemodialysis following ESA therapy. The 1.2% fall in their study was much larger when compared to our results. A plausible explanation is that in contrast to our study, iron therapy was given concurrently which has likely to have potentiated the HbA1c lowering effect reported. A proportion of patients in our study had both therapies and though a similar trend of combined lowering of HbA1c in this group, this failed to reach statistical significance.

Good glycemic control in patients with DM and CKD has been shown to be associated with better survival rates [24]. Proper assessment of glycemic control is therefore vital if this is to be achieved. The results of our study show both statistically and clinically significant falls in the HbA1c following iron and ESA treatment (mean 0.4% following iron and 0.7% following ESA) in the absence of a change in glycemic control.

From a practical view, the data from this study highlights several issues to which diabetes management can be improved in patients with DM and CKD. It shows that HbA1c can be unreliable and can fall following treatment with both iron and ESA therapy. It is essential that healthcare professionals are aware of the potential fluctuations of HbA1c that can occur in this patient group. Alternative methods for measuring glycemic control such as capillary glucose testing and CGMS should be employed and therapy should not be based on the HbA1c value alone. This has particular significance when considering national, international or health service glycemic targets such as the Quality and Outcome Framework (QoF) in the UK which almost exclusively use HbA1c as the sole index by which treatment success is judged.

Glycated albumin has been suggested as an alternative marker to represent glycemic control as it was noted to be similar (in contrast to HbA1c which was higher) in patients with iron deficiency and pre ESA as compared to patients post therapy [8, 20]. Though this may be true, further study is still required and better correlation between glycated albumin and glycemic control is still needed to before this measurement to be more widely used.
The strengths of this study lies in the robust monitoring of glycemic control in patients. 7PGM and CGMS were used in all patients and glycemic control, treatment and HbA1c values were monitored closely. However, this study is limited by its relatively small numbers and though it managed to show that HbA1c values fell both with iron and ESA, there were insufficient numbers to confirm whether the combined effect of both therapies had an added HbA1c lowering effect as compared to a single agent given alone.

Intravenous iron and ESA are increasingly common therapies used in the management of anemia in patients with CKD and DM. The present study has been able to confirm that reported changes in HbA1c following these treatments are indeed independent of changes in glycemic control and so caution is warranted in the interpretation of HbA1c and management of glycemia when based on this measurement alone. At a time when self-monitoring of blood glucose is being discouraged, especially in non-insulin treated patients [25], regular capillary glucose measurements, and the concurrent use of CGMS if available, seems essential in order to accurately assess glycemic control in this group of patients.

**Author contributions:** Dr JM Ng was involved in the study design. He researched and analysed the data and wrote the draft manuscript. He works in the Michael White Research Centre and is employed by the Hull York Medical School, Hull.

Ms M Cooke was responsible for research and discussion of study results. Dr S Bhandari was also involved in the discussion of study results and reviewed the final manuscript. They both work in the renal department in Hull Royal Infirmary, Hull and are employed by the Hull and East Riding NHS trust.

Prof ES Kilpatrick and Prof SL Atkin were involved in designing the study. They reviewed the data, rewrote the manuscript and contributed towards the discussion. Prof ES Kilpatrick works in the department of clinical biochemistry and is employed by the Hull and East Riding NHS trust. Prof SL Atkin works in the Michael White Research Centre and is employed by the Hull York Medical School.

**Declaration of competing interest:** None to declare.

**REFERENCES**

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Table 1. Patients on iron therapy

<table>
<thead>
<tr>
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<th>Before iron mean (95%CI)</th>
<th>After iron Mean (95%CI)</th>
<th>p**</th>
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<tbody>
<tr>
<td>HbA1c (%)</td>
<td>7.40 (6.60,8.19)</td>
<td>6.96 (6.27,7.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>9.71 (9.32,10.05)</td>
<td>10.46(9.97,10.75)</td>
<td>0.001</td>
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<tr>
<td>Hct</td>
<td>0.302(0.285,0.316)</td>
<td>0.334(0.314,0.354)</td>
<td>0.007</td>
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<td>Ferritin (µg/L)</td>
<td>122 (67,176)</td>
<td>307 (211,403)</td>
<td>&lt;0.001</td>
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<tr>
<td>Mean blood glucose (mmol/L)</td>
<td>9.55 (8.20,10.90)</td>
<td>9.71 (8.29,11.13)</td>
<td>0.071</td>
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<tr>
<td>eGFR</td>
<td>34.0(31.9,36.2)</td>
<td>32.8 (30.4,35.2)</td>
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</table>

** paired t test

Table 2. Patients on Erythropoietin Therapy:

<table>
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<tr>
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<th>Before ESA mean (95%CI)</th>
<th>After ESA Mean (95%CI)</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>7.31 (6.42,8.54)</td>
<td>6.63 (6.03,7.36)</td>
<td>0.013</td>
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<td>Hb (g/dl)</td>
<td>9.52 (9.18,9.86)</td>
<td>11.51(11.15,11.85)</td>
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<tr>
<td>Hct</td>
<td>0.324(0.296,0.350)</td>
<td>0.378(0.341,0.398)</td>
<td>&lt;0.001</td>
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<td>Ferritin (µg/L)</td>
<td>344 (241,447)</td>
<td>332 (211,354)</td>
<td>0.37</td>
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<tr>
<td>Mean blood glucose (mmol/L)</td>
<td>8.72 (7.31,10.12)</td>
<td>8.78 (7.47,9.99)</td>
<td>0.893</td>
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<tr>
<td>eGFR</td>
<td>30.5 (28.6,33.4)</td>
<td>31.0 (27.3,33.8)</td>
<td>0.613</td>
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</tbody>
</table>

** paired t test