Venesection is an effective non-pharmacological treatment for patients with high serum ferritin type 2 diabetes

M. Shann Wilson a, b, *, Erik W. Thompson a, c

a Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia
b Pacific Pines Medical Centre, 6/19 Pitcairn Way, Pacific Pines, QLD 4211, Australia
c University of Melbourne, Department of Surgery, St Vincent’s Hospital, Melbourne, Australia

Abstract

Aims/Hypothesis: Previous studies have associated serum ferritin levels with type 2 diabetes, so it’s well recognised that there is a subset of patients with type 2 diabetes and also with elevated serum ferritin. The aim of this pilot study was to test whether venesection is a viable treatment option for patients with type 2 diabetes.

Methods: Four cases with elevated serum ferritin and type 2 diabetes were subjected to monthly venesection for 3–9 months, and monitored 3 monthly for serum ferritin and glycated haemoglobin (HbA1c).

Results: In all four cases, serum ferritin was dramatically reduced, and the HbA1c was reduced in all but one case, which was a diet-only controlled patient with diabetes and had near normal HbA1c. In two cases, HbA1c was reduced by 2–3% units (22–33 mmol/mol), making it superior to any current medication.

Conclusions/Interpretation: Venesection was used here successfully with oral pharmacotherapy, thereby avoiding the use of insulin. Findings of this study may provide clinicians a powerful new tool to intervene in the pathological process of type 2 diabetes by a very simple manoeuvre - venesection. These results need to be repeated and validated in a larger trial as a priority.

1. Introduction

High serum ferritin associated with type 2 diabetes is not recognised as an entity in the current clinical guidelines for the management of type 2 diabetes [1]. In 1997, a cross-sectional population study showed that mildly elevated serum ferritin was associated with elevated fasting serum insulin [2]. This means that people with the higher serum ferritin needed to produce more insulin to remain normoglycemic. In 2008, Le and co-workers suggested that serum ferritin concentration could be used as a predictor for diabetes [3], and a meta-analysis has since showed that elevated levels of serum ferritin may help to identify individuals at risk of type 2 diabetes [4]. In 2002 a study looking at blood letting (venesection) in high ferritin type 2 diabetes demonstrated decreased glycated haemoglobin (HbA1c) levels and changes to both insulin secretion and insulin resistance [5].

Venesection is the treatment of choice for iron overload conditions - haemochromatosis, polycythemia rubra vera, polycythemia cutaneous tarda and clinical iron overload - supported by FerriScan MRI or liver biopsy. Elevated serum ferritin is commonly encountered in general practice and 90% of elevated serum ferritin is due to non-iron overload conditions where venesection is not the treatment of choice. These conditions are chronic alcohol consumption, metabolic syndrome, obesity, diabetes, malignancy, infection and inflammatory conditions [6]. In these conditions serum ferritin ranges between 300 and 1000 µg/l, and in these circumstances the risk of hepatic iron overload is exceedingly low. The high ferritin/iron overload diseases venesection protocol is registered by the Therapeutic Goods Administration (TGA) in Australia (the equivalent of the FDA in the USA) and accessible through the “high ferritin” application hosted by the Australian Red Cross Blood Bank (https://highferritin.transfusion.com.au/).

All this information collectively suggests that while iron overload is virtually unseen in uncomplicated type 2 diabetes, venesection may have important benefits for glucose metabolism, seen as a decrease in HbA1c [5].
This is a preliminary study of high serum ferritin type 2 diabetes that puts the assertion of Goot et al. [6] - “venesection is not the treatment of choice” - to the test. The study design was made with the view that the procedures were first and foremost a clinical treatment protocol for any patient with type 2 diabetes and elevated serum ferritin. The expectation was that venesection would lower the HbA1c, and therefore would be a therapeutic strategy that could be routinely employed in the management of type 2 diabetes in general practice/internal medicine.

Four pilot cases are presented to support the need for further studies to fully establish the utility of venesection as a treatment option in patients with high serum ferritin and type 2 diabetes.

2. Methods

The venesection and all the tests were routinely performed by Queensland Medical Laboratories (QML) Pathology, and the methodology for each test is briefly described below. All patients had monthly venesection and the usual three monthly HbA1c. Monthly venesection was employed as this is the time required for the body to completely replace the blood from the last venesection [7]. Hb was tested at every venesection (and no patients became anaemic) and with the 3 monthly HbA1c. Hb (FBE), serum ferritin, CRP, (to check for distortions due to inflammation), and ANA (to exclude autoimmune conditions) were assayed. All patients were HFE gene screened for haemochromatosis.

2.1. HbA1c was assayed on a COBAS INTEGRA 400/400 plus/800 analyser using COBAS INTEGRA hemolyzing reagent Gen.2 with an anti-coagulated whole blood specimen. All haemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are measured by this assay. The HbA1c results are reported in mmol/mol (as defined by the International Federation of Clinical Chemistry - IFCC), and as a % (as defined by the Diabetes Control and Complications Trial - DCCT) both of which are recorded in this paper in the format mmol/mol (%), for example 42 mmol/mol (6.0%).

2.2. Ferritin was assayed on the ADVIA Centaur Ferritin assay which is a two-site sandwich immunoassay using direct chemi-luminoimetric technology, which uses constant amounts of two anti-ferritin antibodies. The first antibody, in the Lite Reagent, is a polyclonal goat anti-ferritin antibody labeled with acridinium ester. The second antibody, in the Solid Phase, is a monoclonal mouse anti-ferritin antibody, which is covalently coupled to paramagnetic particles.

2.3. C-reactive protein (CRP) was assayed with the wcCRP latex reagent - a suspension of uniform polystyrene latex particles coated with anti-CRP antibody. When serum or plasma containing CRP is added to this reagent a turbidity results due to aggregation of the latex particles. The second antibody, in the Solid Phase, is a monoclonal mouse anti-ferritin antibody, which is covalently coupled to paramagnetic particles.

2.4. CRP was assayed with the same method as described above.

2.5. FBE (full blood examination) that includes Hb (haemoglobin) was assayed with a Sysmex XE-2100 which utilises fluorescent flow cytometry and hydrodynamic focusing technologies to differentiate normal RBC (red blood cells), WBC (white blood cells) and platelet populations.

2.6. HFE genotyping for haemochromatosis (C282Y (c.845G > A), H63D (c.187C > G) and S65C (c.193A > T)) was performed in a NATA accredited QML laboratory by real-time PCR and melt curve analysis using fluorescent resonance energy transfer (FRET) probes.

2.7. Venesection was performed according to a standard protocol with the patient supine, utilising an upper arm vein with a blood pressure cuff applied to 80 mmHg to insert the needle and then reduced to 40 mmHg. The blood collecting bag was placed below the level of the arm to allow the collection of 450 ml of blood. The cuff was deflated, the needle removed, the puncture site dressed, and the blood and bag disposed of into the hazardous waste. This equates to the removal of elemental iron in the order of 250 mg.

3. Results

Throughout the course of this study none of the 4 patients became anaemic (the haemoglobin values were Case 1=124 g/l, Case 2=133 g/l, Case 3=146 g/l and Case 4=136 g/l) or had co-existing clinically significant inflammatory or auto-immune conditions, as measured by CRP and ANA respectively. Three of the 4 cases were heterozygous for the haemochromatosis gene, the details of which will be discussed with each case. Mild iron overload has been demonstrated in the minority of patients with genotypes such as H63D homozygotes or compound heterozygotes (C282Y/H63D). However, with only one copy of a defective gene, serum ferritin is not expected to be elevated above the normal population.

3.1. Case 1 was a 59 year old woman with long-standing diet controlled type 2 diabetes. She was heterozygous for the haemochromatosis (HFE) H63D (c.187C > G) mutation. Her initial HbA1c was 6.5% (47 mmol/mol), but she had been able to keep good control of her diabetes with diet, with her HbA1c ranging between 6.0% (42 mmol/mol) and 6.3% (45 mmol/mol) over the last 3 years. At the time of the first venesection her serum ferritin was 524 µg/L and her HbA1c was 61% (43 mmol/mol) (Fig. 1A). Nine venesections later, her serum ferritin was 52 µg/L and her HbA1c was 6.0% (42 mmol/mol).

3.2. Case 2 was a 47 year old woman who has had type 2 diabetes for at least 5 years and for some of that time was under specialist management. About 2 years prior to this study her HbA1c was at 8.9% (74 mmol/mol) and at the time of the study she was medicated with gliclazide 120 mg (Diamicon MR) and sitagliptin XR 50 mg with metformin 850 mg (Janumet), both in the morning. She was heterozygous for both H63D (c.187C > G) and S65C (c.193A > T) HFE gene mutations. Her initial serum ferritin was 376 µg/L (mildly elevated above the top of the normal range of 320) and her HbA1c was 7.9% (63 mmol/mol). After 6 venesections, her serum ferritin was 71 µg/L and her HbA1c was 6.7% (50 mmol/mol) (Fig. 1B). This is a drop in HbA1c of 1.2% (13 mmol/mol), which is at least equivalent to adding another medication, and was her lowest recorded HbA1c. Ongoing monitoring of these parameters is clearly required for this lady.

3.3. Case 3 was a 52 year old man whose last HbA1c reading a year prior to this study was 6.3% (45 mmol/mol). He was taking alogliptin12.5 mg with metformin1000 mg (NesinaMet) twice daily, and did not have any HFE mutations. At the time of venesection his serum ferritin was 412 µg/L and his HbA1c had jumped to 9.4% (79 mmol/mol). It is important to appreciate that the previous HbA1c was 6.3% (45 mmol/mol), so this was a “recent” elevation and the usual advice here was that he would require insulin therapy from this point on. Instead, 3 venesections later, his serum ferritin was 49 µg/L and his HbA1c had returned to 6.6% (49 mmol/mol) (Fig. 1C).

3.4. Case 4 was a 75 year old man whose type 2 diabetes had been well managed for some time on ‘triple therapy’ of pioglitazone 30 mg (Actos) in the morning and sitagliptin XR 50 mg with metformin 850 mg (Janumet) twice daily with his HbA1c usually under 6.5% (48 mmol/mol). He was heterozygous for the H63D
(c.187C > G) mutation HFE. At the time of the first venesection his serum ferritin was 372 μg/L and his HbA1c was 9.2% (77 mmol/mol). This again was a significant jump over what had been a stable background of effective pharmacological management. After three venesections his serum ferritin was 108 μg/L and his HbA1c was 5.7% (39 mmol/mol) (Fig. 1D).

3.5. As with Case 3, Case 4 experienced an acute deterioration in his type 2 diabetes, despite what had been stable pharmacological management. The three venesections saw this massive drop in HbA1c of 3.5% (38 mmol/mol). Over this period he had put on 4 kg in weight. He had another 4 venesections and his serum ferritin dropped to 57. Nine (9) months after the last venesection his HbA1c was 6.2% (44 mmol/mol). Capillary BGL was not routinely measured for clinical reasons but these results show a good correlation between random glucose concentrations and HbA1c levels at 6.0% (42 mmol/mol).

4. Discussion

To our knowledge, this is the first study to use venesection for the management of type 2 diabetes. Our results have shown that the benefit of venesection may not be restricted to the iron overload diseases, however this certainly requires further corroboration with a larger study. This pilot study clearly demonstrates a measure of reversibility to this progressive disease by modulation of the body’s own physiology/biochemistry rather than with pharmaceutical agents (drugs), in a similar way that weight loss impacts upon type 2 diabetes.

In Case 1 where the diabetes was well controlled with diet alone, venesection had an insignificant impact, or perhaps did stop the progression of her type 2 diabetes. Case 1 effectively became a control subject, showing that monthly serial venesection would not dilute the blood of HbA1c, as her HbA1c never fell below the 6.0% (42 mmol/mol) level. This is not an absolute baseline control, but argues against the conjecture that venesection simply dilutes out the HbA1c and doesn’t change the fundamentals of glycaemic control in the body in this new iron depleted state. It is very important to remember this control as it provides a solid basis for evaluating the results of Cases 2 to 4.

In Case 1 where the diabetes was well controlled with diet alone, venesection had an insignificant impact, or perhaps did stop the progression of her type 2 diabetes. Case 1 effectively became a control subject, showing that monthly serial venesection would not dilute the blood of HbA1c, as her HbA1c never fell below the 6.0% (42 mmol/mol) level. This is not an absolute baseline control, but argues against the conjecture that venesection simply dilutes out the HbA1c and doesn’t change the fundamentals of glycaemic control in the body in this new iron depleted state. It is very important to remember this control as it provides a solid basis for evaluating the results of Cases 2 to 4. In Case 2 where the type 2 diabetes was further developed, the effect of bodily iron depletion was perhaps moderate but nonetheless beneficial. In Case 3 and 4 where the type 2 diabetes had been well managed with the pharmacological agents up to a point when these oral therapies

Fig. 1. Bar graphs depicting the levels of ferritin (solid bars) and glycated haemoglobin (HbA1c) (open bars) for Cases 1–4 (panels A–D, respectively) before and after their series of venesections, as indicated.
“suddenly” failed, venesection produced dramatic results. Case 3 experienced a huge drop in HbA1c of 2.8% (31 mmol/mol), and Case 4 had a drop of HbA1c 3.5% (38 mmol/mol) with no adverse side effect other than the inconvenience and the discomfort of the venesection procedures. To their relief, therapy with insulin was put off for now, and hopefully forever. It seems likely that in these patients at this point in their disease, body iron overload was pivotal to the pathological progression of their type 2 diabetes. Excess iron has been shown to induce diabetes in animal models, where higher heme iron intake and increased body iron stores were significantly associated with a greater risk of type 2 diabetes [9]. In the ob/ob mouse model of type 2 diabetes, dietary iron restriction or iron chelation protects from diabetes and loss of beta-cell function [10]. In rat liver and human HepG2 hepatocytes, iron depletion by deferoxamine up-regulates glucose uptake and insulin signalling [11]. There is the question of what constitutes normal iron stores and normal serum ferritin. Case 4 had a dramatic response with his serum ferritin at 108 μg/L, so the upper end of the normal range at 320 μg/L may need to be re-evaluated in regards to the effect of this “subtle” iron overload on glycaemic control. The take home point from this study is the total body depletion of iron stores with venesection, monitored with serum ferritin, generates a significant improvement in glycaemic control and thus an improvement in type 2 diabetes.

While these results alone may be sufficient for the clinician, the underlying pathobiology warrants further research. The link between high serum ferritin, and thus the presumption that iron stores are increased, and the impairment of glycaemic control has been recognised and studied for more than the past 2 decades. In addition, the correlation between elevated serum ferritin has been made with a number of disorders where glucose metabolism is impaired. This effect for high serum ferritin type 2 diabetes was elegantly demonstrated in a 2002 study where these effects were mediated through both insulin secretion and insulin resistance [5]. To the best of our knowledge, there are no quantitative studies that define the relative contributions of the components of impaired glycaemic control, but fortunately there are a lot that describe the various individual impediments.

From the clinical perspective, we make the assumption that by the time the diagnosis of type 2 diabetes is made, half the beta-cell mass has been lost and we are dealing with a progressive and incurable disease [12]. It has been shown that beta cells are exquisitely vulnerable to oxidative stress leading to cell death, and hence catalytically active iron [13]. Thus if we take the beta-cells as the weak link in the process then the damage to the beta-cells from accumulated iron may occur by the Fenton reaction which generates hydroxyl (HO.) and the hydroperoxyl (HOO.) radicals as follows. (1) Fe2+ + H2O2 → Fe3+ + HO· + OH· and (2) Fe3+ + H2O2 → Fe2+ + HOO· + H+. (http://en.wikipedia.org/wiki/Fenton%27s_reagent, [14]) The hydrogen peroxide is generated by the mitochondria [15] and the free radicals so generated act as powerful, non-selective oxidants causing intercellular damage, and presumably cell death over time. The physiological and biochemical robustness of liver and muscle could lead to the assumption that these are less important, however the finding that insulin resistance is improved with iron depletion indicates that glucose metabolism is also affected at these sites [5]. Electron microscopy has shown that iron deposits were restricted to beta-cells with progressive loss of their endocrine granules [16]. It has also been shown that iron accumulation may be responsible for the impaired insulin effect and may cause impaired hepatic insulin extraction [17]. Therefore, while the beta-cells are the likely weak link, there is a body-wide impairment of glucose metabolism involving beta-cells, liver and muscle.

It is clear that iron depletion positively impacts upon type 2 diabetes, but it is important that glucose metabolism in other scenarios is also considered, both to support the findings and conclusions above, but also to guide research on the effects of iron depletion in these other circumstances and disease states. In contradistinction to the clinical guidelines, understanding the role of functionally significant iron overload as measured by serum ferritin in the scientific literature is replete. Epidemiologists in 2005 observed that blood donation is associated with decreased risk of type 2 diabetes (and cardiovascular disease). Blood donation is simultaneously associated with increased insulin sensitivity and decreased iron stores, which seems to impact on insulin action, even in healthy people [18]. The Camden Study published in 2006 found a correlation between elevated serum ferritin concentrations for pregnant women early in gestation and an increased risk of gestational diabetes, in part mediated by maternal fat mass and obesity [19]. A 2009 study identified that insulin resistance correlated with ferritin levels in premenopausal women [20]. A 2014 meta-analysis of observational studies found that increased ferritin levels are independently and positively associated with metabolic syndrome, also termed pre-diabetes [21]. Iron has been shown to be a risk factor for metabolic syndrome, and phlebotomy of humans with impaired glucose tolerance and ferritin at the highest quartile of normal showed improved glucose tolerance [22]. In addition it has been shown that increased ferritin levels are associated with the metabolic insulin resistance syndrome [23], and in patients with metabolic syndrome venesection resulted in improvements in markers of glycaemic control [24].

The intention of this study was to verify that venesection had a place in the management of type 2 diabetes in the 21st century. At the very least it identifies the need for further study. It is imperative that we widen the net to include women with gestational diabetes, patients with metabolic syndrome, children with type 2 diabetes, indigenous populations that may have a predisposition for type 2 diabetes (as is the case with the Australian Aboriginal), patients taking the atypical antipsychotics and even “apparently normal” individuals with elevated serum ferritin. If we take the view that moderately elevated serum ferritin, indicative of subtle iron overload, is one of the pivotal steps in the pathogenesis of type 2 diabetes, then it might be wise to start iron depletion therapy by venesection as soon as elevated serum ferritin is indicated. We may be able to catch type 2 diabetes before half the beta-cells are lost. If venesection proved a useful therapeutic tool, then the cost savings on the oral anti-diabetic agents could be substantial to patients and governments across the world. Another aspect for further action/study would be to have a monthly registered venesection schedule for type 2 diabetic patients with the blood banks, and for this to become routine practice. In this study all the blood that was taken was discarded because there was no recognised protocol for monthly venesection, as is the case for the iron overload diseases as discussed above. While managing our patients with type 2 diabetes with venesection, we could also be providing blood banks with much needed stocks.

Funding

This pilot study was not funded. All tests were routine and ordered at no cost to the patient.

Conflict of interest

Both authors have no known conflicts of interest that might bias this work. Full acknowledgment is given in the manuscript to all the people who supported us in this work. Dr Wilson provided the conception of study and design, acquisition of data, analysis and interpretation of data; drafting the article and revising it critically.
for important intellectual content. Professor Thompson made substantial contributions to interpretation of data; drafting the article and revising it critically for important intellectual content. Both authors gave final approval of the version to be published.

Acknowledgments

The authors gratefully acknowledge the patient subjects who participated in this study, Dr Charles Appleton from QML for provision of the methods used, and Associate Professor Elizabeth Williams for assistance with the figures.

References