Advances in Medical Sciences xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Endothelial function, antioxidant status and vascular compliance in newly diagnosed *HFE* C282Y homozygotes

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ARTICLE INFO

Article history:
Received 16 January 2013
Accepted 1 July 2013
Available online xxx

Keywords:
Antioxidant
Compliance
Haemochromatosis
Vascular

ABSTRACT

Purpose: This pilot study was aimed to establish techniques for assessing and observing trends in endothelial function, antioxidant status and vascular compliance in newly diagnosed *HFE* haemochromatosis during the first year of venesection.

Patients/methods: Untreated newly diagnosed *HFE* haemochromatosis patients were tested for baseline liver function, iron indices, lipid profile, markers of endothelial function, anti-oxidant status and vascular compliance. Following baseline assessment, subjects attended at 6-weeks and at 3, 6, 9 and 12-months for follow-up studies.

Results: Ten patients were recruited (M = 8, F = 2, mean age = 51 years). Venesection significantly increased high density lipoproteins at 12-months (1.25 mmol/L vs. 1.37 mmol/L, $p = 0.01$). However, venesection did not significantly affect lipid hydroperoxides, intracellular and vascular cell adhesion molecules or high sensitivity C-reactive protein (0.57 μmol/L vs. 0.51 μmol/L, $p = 0.45$, 427.4 ng/ml vs. 307.22 ng/ml, $p = 0.54$, 517.70 ng/ml vs. 377.50 ng/ml, $p = 0.51$ and 290.75 μg/dL vs. 224.26 μg/dL, $p = 0.25$). There was also no significant effect of venesection on anti-oxidant status or pulse wave velocity (9.65 m/s vs. 8.74 m/s, $p = 0.34$).

Conclusions: Venesection significantly reduced high density lipoproteins but was not associated with significant changes in endothelial function, anti-oxidant status or vascular compliance. Larger studies using this established methodology are required to clarify this relationship further.

1. Introduction

Iron overload in *HFE* haemochromatosis causes deranged liver enzymes. Venesection is the optimal treatment for *HFE* haemochromatosis where improvement of liver enzymes and reversal of hepatic fibrosis have been reported [1,2]. Elevated iron levels in *HFE* haemochromatosis patients are known to be associated with increased oxidative stress. Kom et al. [3] demonstrated increased urinary isoprostanes and reduced vitamin A levels in iron overloaded *HFE* haemochromatosis patients. Following venesection and normalisation of iron levels, they reported a significant reduction in urinary isoprostanes (245 pg/mg creatinine vs. 146 pg/mg creatinine, $p < 0.001$) and a significant increase in vitamin A (0.34 μg/ml vs. 1.36 μg/ml, $p = 0.035$) suggesting a reduction in oxidative stress [3].

Norris et al. [4] provided evidence of endothelial dysfunction in *HFE* haemochromatosis. Their comparison of adhesion molecule expression between 139 subjects with *HFE* C282Y haemochromatosis and 27 healthy controls identified significantly higher sICAM ($p = 0.0059$) and E-selectin ($p = 0.0006$) as well as significantly lower L-selectin ($p = 0.0002$) levels in *HFE* haemochromatosis. Other researchers have also associated elevated iron and reductions in ascorbate in *HFE* haemochromatosis patients with an improvement in ascorbate noted following venesection [5,6].

This decrease in isoprostane production combined with an improved anti-oxidant status following venesection suggests an overall reduction in lipid peroxidation and antioxidant consumption following vensection. This pilot study was aimed to establish

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http://dx.doi.org/10.1016/j.advms.2013.07.001

techniques for assessing and observing trends in serological endothelial function, antioxidant status and vascular compliance in newly diagnosed *HFE* haemochromatosis subjects during the first year of venesection.

2. Patients and methods

2.1. Patient recruitment

Newly diagnosed *HFE* haemochromatosis patients, between the ages of 20 and 75, who had not previously undergone venesection, were recruited. *HFE* haemochromatosis was defined by the phenotypic pattern of raised ferritin (at time of recruitment or at some time in the past) in combination with homozygosis for *HFE* C282Y. Patients with known hypertension (blood pressure >160/90 mm Hg), diabetes mellitus, a history of cardiovascular disease and those taking lipid lowering agents or hormonal preparations were excluded. Written informed consent was obtained.

2.2. Clinical intervention – venesection

Following initial assessment, patients were enrolled in a standard venesection programme where fortnightly phlebotomy of one unit of whole blood (approx. 400 ml) was performed. At the time of this study the target ferritin and iron saturations of the initial phase of the venesection programme were 100 μg/L and 50% respectively. Fortnightly venesection continued until these targets were achieved then the venesection interval increased to three monthly.

2.3. Patient assessment

2.3.1. Baseline clinical parameters

Following completion of initial baseline examinations and investigations at recruitment, patients subsequently attended at 6-weeks and at 3, 6, 9 and 12-months for follow-up. Blood pressure (mm Hg) and body mass index [BMI = weight (kg) divided by height (metres) squared] were recorded after an overnight fast followed by serological assessment of endothelial dysfunction and anti-oxidant status.

2.4. Biochemical assessment

2.4.1. Blood sampling

Fasting peripheral venous blood samples for plasma glucose, serum lipid profiles, liver function tests and iron studies were performed routinely at each assessment time-point. For serological assessment of endothelial dysfunction and anti-oxidant status, plasma samples for ascorbate were centrifuged immediately while serum collected for all other assays was clotted for 15 min and then centrifuged. All samples were transferred to 2 ml tubes (Sarstedt, Ireland) and stored at −80 °C. All commercial assay analyses were performed according to the manufacturers' guidelines. Intra- and inter-assay coefficients of variation were within satisfactory limits according to the manufacturers' guidelines.

2.4.2. Plasma lipid hydroperoxides

These were measured spectrophotometrically using the Ferrous Oxidation-Xylenol Orange-version 1-assay (FOX 1) which was used to determine hydroperoxides (HPO) in the aqueous phase of serum. Hydroperoxides oxidise ferrous ions to ferric ions in dilute acids and the resultant ferric ions were then determined using ferric sensitive dyes as an indirect measure of hydroperoxide concentration.

2.4.3. Intracellular and vascular cell adhesion molecules

Plasma soluble intercellular adhesion molecule-1 (sICAM-l) and soluble vascular cell adhesion molecule-1 (sVCAM-l) were measured using commercially available ELISA kits from Eli-pair (Diaclone, Besançon, France).

2.4.4. Ascorbate

Concentrations were determined by the enzymatic oxidation of ascorbic acid and subsequent quinoxaline formation to generate a fluorescent derivative measured on the Cobas Fara centrifugal analyser as described by Vuilleumier et al. [7].

2.4.5. Lipid soluble antioxidants

These levels were measured using a high performance liquid chromatography technique (HPLC) using diode array detection to assess retinol, γ-tocopherol, α-tocopherol, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene and lycopene according to the method of Craft and Soares [8]. The detection limits for retinol and the tocopherols were 0.05 nmol/l while 0.005 nmol/l was used for carotenoids.

2.4.6. High-sensitive C-reactive protein

This was measured with a latex-enhanced immunoturbidimetric assay (Randox Pharmaceuticals, Curmlin) using an ILab 600 biochemical analyzer and ILab 600 computer software (Instrumentation Laboratories, Warrington).

2.5. Estimation of vascular compliance

The methodologies of pulse wave analysis (PWA) and pulse wave velocity (PWV) gated to the cardiac cycle have previously been described [9–11]. After rest in the supine position in a temperature controlled room for a minimum of 15 min, radial pulse wave analysis was recorded with a Millar tonometer and the Sphygmocor system model SCORPx, incorporating the pulse wave velocity system Model SCOR-Vx (SPC-301; Millar instruments and Atcor medical, Sydney, Australia). For PWA, triplicate measurements were made in the supine position from the radial artery of the dominant arm and the average calculated. The Sphygmocor analysis software automatically processed the radial artery waveform data and using a generalised transfer function generated measures of vascular compliance including augmentation index calibrated to 75 beats per minute (Aglx75), time to reflectance (TR), Buckberg's subendocardial viability ratio (SEVR) and ejection duration percentage (ED%). Calculation of PWV was similar to PWA with the analysis gated to the cardiac cycle with separate readings from the dominant radial artery (distal site) and ipsilateral carotid artery (proximal site). Carotid-radial PWV was measured rather than carotid-femoral due to ease of reproducibility and acceptability to patients.

2.6. Statistical analysis

Analyses were performed using the Statistical Programme for Social Sciences (SPSS 15.0 for windows; SPSS Inc., Chicago, IL, USA). Due to the small number of subjects, non-parametric analysis was undertaken with Friedman repeated measure analysis. Where the Friedman test demonstrated significance ($p < 0.05$) or where trends were observed, further analysis with Wilcoxon signed-rank test was performed to establish when the difference occurred during the study period.

3. Results

3.1. Patient demographics

Ten patients with *HFE* haemochromatosis were recruited (male = 8, female = 2, mean age = 51 ± 13 years, mean systolic blood pressure (SBP) = 145 ± 20, mean diastolic blood pressure

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(DBP) = 87 ± 10 and mean BMI = 29 ± 6) None of the patients were smokers.

3.2. Clinical evaluation

A significant reduction in mean SBP was identified at nine months following venesection (Baseline: 145 ± 20 mm Hg vs. 9 months: 131 ± 22 mm Hg, $p = 0.02$), However, SBP at study completion remained unchanged from baseline. Mean DBP were unaffected by venesection (Table 1). No significant change in BMI was noted (Baseline: 29 ± 6 vs. 12 months: 30 ± 5, $p = 0.80$).

3.3. Haematological and biochemical assessment

Fasting glucose levels were not affected by venesection (Baseline: 5.7 ± 1.4 mmol/L vs. 12 months: 5.5 ± 1.1 mmol/L, $p = 0.89$). Although venesection had a positive impact on high density lipoprotein (HDL) (Baseline: 1.3 ± 0.3 mmol/L vs. 12 months: 1.4 ± 0.3 mmol/L, $p = 0.01$), there was no significant effect on total cholesterol, low density lipoprotein (LDL) or triglyceride levels (Table 1).

Vensection had a positive effect on liver biochemistry with significant reductions in alanine transaminase (ALT) identified as early as six weeks (Baseline: 64 ± 46 U/L vs. 6 weeks: 50 ± 34 U/L, $p = 0.01$) and continued improvements at twelve months in ALT (Baseline: 64 ± 46 U/L vs. 12 months: 30 ± 15 U/L, $p = 0.001$) and aspartate transaminase (AST) (Baseline: 47 ± 24 U/L vs. 12 months: 30 ± 11 U/L, $p = 0.05$) (Table 1).

Ferritin (Baseline: 1161 ± 657 ng/ml vs. 12 months: 159 ± 328 ng/ml, $p < 0.001$) and transferrin saturation (Baseline: 85 ± 20% vs. 43 ± 25%, $p = 0.005$) were significantly reduced after twelve months of venesection compared to baseline. While total iron binding capacity (TIBC) (Baseline: 43 ± 5 μg/L vs. 12 months: 54 ± 8 μg/L, $p = 0.002$) significantly increased compared to baseline. Whilst there was a reduction in serum iron levels, it was not statistically significant (Baseline: 37 ± 11 μmol/L vs. 12 months: 22 ± 11 μmol/L, $p = 0.14$) (Table 1).

3.4. Endothelial function

3.4.1. Lipid hydroperoxides

There was no significant difference in lipid hypoperoxides over the study period (Baseline: 0.6 ± 0.1 μmol/L vs. 12 months: 0.5 ± 0.1 μmol/L, $p = 0.45$) (Table 1).

Table 1
Analysis of clinical and haematological parameters, serological endothelial function, anti-oxidant status, high sensitive C-reactive protein and vascular compliance using the Friedman test. Results are presented as mean and standard deviations.

	Assessment time-point						
Parameter	Baseline	6 weeks	3 months	6 months	9 months	12 months	*p*-Value
SBP (mm Hg)	145.40 (19.87)	141.20 (18.75)	138.20 (19.93)	137.00 (18.08)	131.40 (29.08)	146.20 (22.01)	0.08
DBP (mm Hg)	87.20 (10.18)	84.00 (10.11)	85.60 (6.80)	83.65 (6.89)	81.90 (8.71)	87.90 (11.72)	0.39
Glucose (mmol/L)	5.67 (1.36)	5.78 (0.95)	5.42 (1.14)	5.37 (1.23)	5.53 (1.01)	5.47 (1.06)	0.89
Cholesterol (mmol/L)	4.61 (1.14)	4.49 (0.98)	4.66 (0.88)	4.71 (0.93)	4.75 (1.02)	4.82 (1.00)	0.66
HDL (mmol/L)	1.25 (0.27)	1.30 (0.32)	1.32 (0.31)	1.40 (0.32)	1.37 (0.33)	1.37 (0.34)	0.01
LDL (mmol/L)	2.59 (1.00)	2.41 (0.95)	2.48 (0.79)	2.60 (0.98)	2.64 (0.87)	2.69 (1.08)	0.95
HDL/LDL Ratio	3.82 (1.19)	3.65 (1.20)	3.73 (1.25)	3.51 (1.05)	3.63 (1.08)	3.78 (1.45)	0.36
Triglycerides (mmol/L)	1.72 (1.01)	1.75 (1.34)	1.91 (1.28)	1.56 (0.98)	1.64 (1.03)	1.68 (0.81)	0.78
Iron (μmol/L)	36.60 (10.05)	32.90 (12.07)	31.20 (17.22)	31.20 (17.70)	24.15 (14.47)	22.30 (11.42)	0.14
Ferritin (ng/ml)	1161.30 (656.74)	844.60 (899.27)	570.60 (497.46)	356.40 (389.42)	433.35 (1002.51)	158.50 (328.35)	<0.001
Transferrin saturation (%)	85.37 (19.88)	75.32 (23.36)	66.74 (30.56)	62.44 (31.89)	48.05 (28.85)	42.60 (25.44)	0.005
TIBC (μg/L)	43.00 (5.42)	43.50 (6.08)	45.00 (8.84)	48.60 (10.19)	50.60 (5.76)	54.10 (7.87)	0.002
Bilirubin (μmol/L)	13.70 (6.45)	11.50 (4.48)	10.65 (7.39)	11.10 (4.53)	11.10 (3.41)	12.30 (4.81)	0.09
Albumin (g/L)	45.80 (3.01)	45.10 (1.52)	46.15 (3.45)	46.60 (0.97)	46.30 (1.70)	45.30 (3.47)	0.15
ALP (U/L)	79.20 (20.12)	77.80 (17.18)	75.40 (17.99)	74.50 (19.74)	84.10 (41.68)	86.30 (32.67)	0.49
ALT (U/L)	63.90 (45.52)	49.90 (34.41)	41.00 (26.67)	31.00 (10.03)	48.80 (69.34)	30.00 (15.00)	0.001
AST (U/L)	46.80 (24.09)	41.50 (20.71)	38.30 (19.86)	32.50 (8.64)	42.10 (42.80)	30.30 (10.80)	0.05
γGT (U/L)	98.50 (104.49)	74.50 (84.43)	75.70 (88.75)	75.55 (84.95)	146.90 (258.88)	65.10 (75.38)	0.09
HPO (μmol/L)	0.57 (0.14)	0.55 (0.17)	0.53 (0.22)	0.51 (0.16)	0.47 (0.09)	0.51 (0.09)	0.45
sICAM1 (ng/ml)	427.40 (182.61)	313.90 (119.46)	395.60 (174.11)	399.05 (238.72)	361.00 (108.27)	307.22 (122.12)	0.54
sVCAM1 (ng/ml)	517.70 (192.87)	407.00 (148.24)	534.40 (257.70)	525.90 (288.96)	488.40 (187.01)	377.50 (119.79)	0.51
Vitamin C (μmol/L)	67.46 (34.91)	66.35 (36.01)	66.38 (44.95)	76.39 (59.03)	83.42 (43.10)	77.76 (52.21)	0.95
α-Carotene (μmol/L)	0.07 (0.05)	0.06 (0.03)	0.04 (0.03)	0.05 (0.04)	0.06 (0.06)	0.06 (0.05)	0.94
α-Tocopherol (μmol/L)	26.15 (6.46)	25.82 (6.83)	25.65 (6.28)	24.45 (4.37)	25.84 (7.63)	25.62 (6.63)	0.94
β-Carotene (μmol/L)	0.23 (0.12)	0.21 (0.10)	0.21 (0.18)	0.21 (0.15)	0.23 (0.16)	0.25 (0.19)	0.97
β-Cryptoxanthin (μmol/L)	0.06 (0.04)	0.06 (0.04)	0.06 (0.05)	0.07 (0.07)	0.07 (0.06)	0.06 (0.04)	0.56
γ-Tocopherol (μmol/L)	2.92 (1.06)	3.11 (1.46)	3.02 (1.17)	2.72 (1.02)	2.85 (1.15)	2.55 (1.29)	0.51
Lutein (μmol/L)	0.12 (0.04)	0.10 (0.03)	0.11 (0.05)	0.10 (0.04)	0.11 (0.03)	0.10 (0.05)	0.88
Lycopene (μmol/L)	0.53 (0.35)	0.43 (0.24)	0.36 (0.20)	0.61 (0.55)	0.57 (0.41)	0.74 (0.68)	0.23
Retinol (μmol/L)	1.66 (0.75)	1.66 (0.69)	1.34 (0.79)	1.30 (0.64)	1.81 (0.84)	1.56 (0.47)	0.22
Zeaxanthin (μmol/L)	0.03 (0.01)	0.02 (0.01)	0.03 (0.03)	0.03 (0.02)	0.03 (0.02)	0.03 (0.02)	0.82
hsCRP (μg/dL)	290.75 (263.94)	232.27 (199.07)	258.42 (197.43)	344.47 (620.98)	536.44 (1050.35)	224.26 (170.30)	0.25
AgIx75 (%)	19.77 (11.63)	17.43 (11.99)	15.58 (15.20)	17.55 (13.38)	19.08 (11.83)	16.37 (14.08)	0.22
TR (msec)	145.60 (12.05)	145.48 (8.92)	146.77 (13.45)	143.47 (14.04)	145.33 (16.02)	142.65 (6.48)	0.95
ED (%)	35.72 (3.23)	36.35 (5.09)	35.42 (3.41)	33.57 (3.51)	35.05 (4.21)	37.02 (5.57)	0.28
SEVR (%)	153.38 (16.22)	150.88 (27.70)	159.58 (21.34)	170.93 (24.52)	159.68 (22.33)	147.38 (28.64)	0.21
PWV (m/s)	9.65 (2.35)	9.03 (2.69)	8.85 (1.40)	9.00 (1.17)	9.22 (0.96)	8.74 (1.16)	0.34

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate transaminase; DBP, diastolic blood pressure; GGT, γ-glutamyl transpeptidase; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; TIBC, total iron binding concentration, hsCRP, high sensitive C-reactive protein; HPO, hydroperoxides; sICAM1, soluble intracellular adhesion molecule-1; sVCAM1, soluble vascular cell adhesion molecule-1, AgIx75, augmentation index calibrated to 75 beats per minute; ED, ejection duration; PWV, pulse wave velocity; SEVR, subendocardial viability ratio; TR, time to reflectance.

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3.4.2. Cell adhesion molecules

There was no significant change in sICAM (Baseline: 427 ± 183 ng/ml vs. 12 months: 307 ± 122 ng/ml, $p = 0.54$) and sVCAM (Baseline: 518 ± 193 ng/ml vs. 12 months: 378 ± 120 ng/ml, $p = 0.51$) (Table 1).

3.5. Antioxidant status

There was no significant alteration in anti-oxidant status over the 12 month study period. Vitamin C (Baseline: 68 ± 35 μmol/L vs. 12 months: 78 ± 52 μmol/L, $p = 0.95$), β-carotene (Baseline: 0.2 ± 0.1 μmol/L vs. 12 months: 0.3 ± 0.2 μmol/L, $p = 0.97$) and lycopene levels (Baseline: 0.5 ± 0.4 μmol/L vs. 12 months: 0.7 ± 0.7 μmol/L, $p = 0.23$) were not significantly increased from baseline (Table 1).

3.6. High sensitive C-reactive protein

There was no significant change in hsCRP levels at twelve months (Baseline: 291 ± 264 μg/dL vs. 12 months: 224 ± 170 μg/dL, $p = 0.25$) (Table 1).

3.7. Vascular compliance

Venesection did not significantly alter PWV (Baseline: 10 ± 2 m/s vs. 12 months: 9 ± 1 m/s, $p = 0.34$), AgIx75 (Baseline: 19.8 ± 11.6% vs. 12 months: 16.4 ± 14.1%, p = 0.22), SEVR (Baseline: 153 ± 16% vs. 12 months: 147 ± 29%, $p = 0.21$) and TR (146 ± 12 ms vs. 143 ± 7 ms, $p = 0.95$) (Table 1).

4. Discussion

Increased cardiovascular risk has previously been linked to an elevation in body iron stores particularly from research performed in the Finnish population who are known to have a high incidence of *HFE* haemochromatosis [12–18]. It has also been suggested that elevated ferritin levels identified in *HFE* haemochromatosis can lead to iron deposition and cardiovascular complications [19–21]. In addition, Ellervik et al. [22] have recently reported the association of the *HFE* C282Y haemochromatosis genotype, elevated transferrin saturation and an increased risk of antihypertensive use.

The strongest arguments against this association have come from a large individual patient meta-analysis by van der A et al. [23] that excluded increased cardiovascular risk in individuals heterozygous for haemochromatosis and an autopsy study by Miller et al. [24] that suggested a degree of cardioprotection in those with hemochromatosis associated with HFE C282Y homozygosity. The latter has recently been explained by mutational effect of selective iron depletion of the macrophage, a key cell type in atherogenesis [25,26].

It is currently unclear if venesection reduces cardiovascular risk. Zacharski et al. [27,28] acknowledged a possible trend towards improved outcomes particularly in younger patients when assessing the effects of six-monthly venesection on cardiovascular outcomes in symptomatic peripheral vascular disease. However, they reported no significant decrease in all-cause mortality, myocardial infarction and stroke.

Whilst the potential mechanism for advantageous haemodynamic effects remains unclear following venesection, some postulate that observed differences may reflect the enhanced effect of HDL on vascular endothelium via reduced LDL oxidation or through cholesterol reabsorption [29]. Others have reported a relationship between excess iron level, lipid homeostasis and cardiovascular risk. Van Jaarsveld and Pool [30] demonstrated a 7% higher HDL level in healthy individuals following blood donation whilst Sultana et al. [31] identified a positive correlation between total iron binding capacity and HDL levels in patients who suffered acute myocardial infarction. This improvement in HDL levels could have significant clinical implications particularly as Rubins et al. [32] revealed that a 7.5% increase in HDL resulted in a 22% reduction in coronary events in males with known cardiovascular disease.

Our study identified that iron reduction in *HFE* haemochromatosis was associated with a significant 9.6% rise in HDL. However, it did not otherwise affect the lipid profile and targeting HDL level alone does not appear to be beneficial in reducing cardiovascular risk. In a recent trial of patients who had had a recent acute coronary syndrome, treatment with dalcetrapib markedly increased HDL cholesterol levels (31–40%) compared to placebo (4–11%) but did not reduce LDL cholesterol level or the risk of recurrent cardiovascular events [33]. Despite this result, new research has suggested that importance of HDL particle number in cardiovascular outcome and highlights that manipulation of HDL may still have important clinical applications in this setting [34,35].

Given the positive changes in HDL with vensection, it was postulated that further advantageous effects may include improvements in endothelial function, anti-oxidant status and vascular compliance. However, contrary to some published evidence, no significant effect on these parameters was observed in this study. Gaenzer et al. [18] previously found a correlation between iron and thiobarbituric acid-reactive substance (TBARS). Although TBARS is a less specific assessment of lipid peroxidation than the FOX 1 assay, their TBARS results were supported by measurement of glutathione levels and suggest that oxidative stress was at least indirectly related to iron [36]. Furthermore Houglum et al. [37] identified higher oxidatively modified proteins (carbonyl groups) in untreated *HFE* haemochromatosis patients compared to controls (110 nmol/μL vs. 53 nmol/μL, $p < 0001$), which following venesection decreased significantly compared to untreated *HFE* C282Y haemochromatosis patients (66 nmol/μL vs. 110 nmol/μL, $p < 0001$).

Shizukuda et al. [38] measured oxidative stress-related biochemical markers in three asymptomatic age- and gender-matched groups including newly diagnosed *HFE* haemochromatosis patients ($n = 22$), *HFE* haemochromatosis subjects who had undergone at least six months of venesection ($n = 21$) and controls ($n = 21$). Newly diagnosed *HFE* haemochromatosis patients had a significant decline in markers of oxidative stress (erythrocyte glutathione: 6.8 μmol/gHb vs. 4.1 μmol/gHb, $p < 0.001$; plasma myeloperoxidase: 27.3 ng/ml vs. 14.3 ng/ml, $p < 0.05$) following the completion of six months of venesection therapy. However, *HFE* C282Y haemochromatosis patients with more than six months of previous venesection demonstrated significant elevations in oxidative stress markers compared with controls (erythrocyte glutathione: 8.3 μmol/gHb vs. 3.6 μmol/gHb, $p < 0.05$; plasma lipid peroxidation: 3.5 ng/ml vs. 2.0 ng/ml, $p < 0.05$; plasma myeloperoxidase: 69.8 ng/ml vs. 4.6 ng/ml, $p < 0.05$). Their data may suggest that initial, intensive phlebotomy therapy attenuates oxidative stress in *HFE* haemochromatosis subjects but that oxidative stress may persist or rebound during the maintenance phase of venesection. In the same cohort the authors found that venesection did not improve left ventricular systolic function as determined by exercise echocardiography and electrocardiography but strain rate, a sensitive echocardiography derived measure of diastolic function, did appear to correlate with the above biomarkers of oxidative stress [39,40].

Previous research from our own group has demonstrated that at various stages of treatment, *HFE* haemochromatosis patients when compared to healthy controls have significantly diminished levels of Vitamin C (51.3 μmol/L vs. 89.1 μmol/L, $p = 0.013$), retinol (1.78 μmol/L vs. 2.46 μmol/L, $p = 0.001$) and alpha-tocopherol (5.91 μmol/L vs. 7.24 μmol/L, $p = 0.001$).[6] Assessment of patients attending a *HFE* haemochromatosis venesection programme in this current study afforded an opportunity to determine the effects of treatment over a period of time rather than at a solitary time-point as reported in our previous study and found no change in anti-oxidant

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status [6]. Previous literature regarding venesection in patients with iron overload as described by Brissot et al. [41] has shown an increase in Vitamin C in 67 venesection treated *HFE* haemochromatosis patients (untreated: 19.5 μg/10^8 WBC vs. treated 34.3 μg/10^8 WBC). Despite published evidence supporting increased levels of vitamin C following venesection in iron overload, van Jaarsveld and Pool [30] discovered a significant reduction in vitamin C levels when 23 healthy males donated 500 ml of blood on three occasions within six weeks (28.58 μM vs. 8.59 μM, $p = 0.0347$). Therefore if venesection is associated with improvement in anti-oxidant status it may be limited to individuals with iron overload.

Endothelial dysfunction has long been recognised as an important early functional abnormality and accepted surrogate marker of atherosclerosis while hsCRP is a predictor of cardiovascular events and mortality [42–45]. Such reductions in vascular compliance detected by elevated PWV combined with associated serological endothelial dysfunction suggest increased cardiovascular risk [46]. Kiechl et al. [14] previously suggested an association between serum ferritin concentrations and progression of carotid atherosclerosis in the general population. More recently Gaenzer et al. [18] reported a relationship between endothelial dysfunction, detected by a reduction in endothelium-dependent dilation (EDD) of the brachial artery, and increased intima-media thickness (IMT) of the carotid artery when male *HFE* haemochromatosis patients receiving venesection were compared with age-matched controls. They also identified that when previously untreated male *HFE* haemochromatosis patients were re-investigated after venesection, a significant improvement in EDD was observed (2.6% vs. 5.5%, $p = 0.0015$).

In keeping with a non-significant change in arterial compliance in our study, others suggest the absence of any relationship between serum iron and vascular compliance. Vergnaud et al. [47] reported that there was no association between baseline serum ferritin levels, iron intake levels at baseline and subsequent PWV after 7.5 years of follow-up. They also reported no relationship between iron profiles, carotid artery IMT or presence of carotid plaques. The majority of other studies in the general population concur with the absence of such a relationship [14,48–51]. On the other hand, it is important to note that Vergnaud et al. [47] excluded patients with *HFE* C282Y haemochromatosis and our study is only the second study that has assessed vascular compliance specifically in *HFE* haemochromatosis.

Given that previous researchers have described a relationship between body iron stores, endothelial dysfunction and overall cardiovascular risk it is suggested that further larger prospective studies to evaluate the impact of venesection on serological endothelial dysfunction, antioxidant status and vascular compliance in *HFE* haemochromatosis patients are required [12–18].

5. Conclusions

Vensection is in *HFE* haemochromatosis correlates with an early and sustained improvement in HDL. Such an improvement does not appear to be related to significant improvements in serological endothelial function, antioxidant status and arterial compliance. However, larger studies using this established methodology are required to clarify this relationship further.

Conflict of interests

None.

Financial disclosure

The authors greatly acknowledge the financial support of The Royal Victoria Hospital Liver Support Group.

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