9-18-2020

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10.1080/03009742.2020.1800081

This is an Accepted Manuscript of an article published by Taylor & Francis in SCANDINAVIAN JOURNAL OF RHEUMATOLOGY on 18/09/2020, available online: http://www.tandfonline.com/10.1080/03009742.2020.1800081

Rehman, A., Carroll, G. J., Powell, L. W., Ramm, L. E., Ramm, G. A., & Olynyk, J. K. (2020). Arthropathy in hereditary haemochromatosis segregates with elevated erythrocyte mean corpuscular volume. *Scandinavian Journal of Rheumatology, 50*(2), 139-142. https://doi.org/10.1080/03009742.2020.1800081

Arthropathy in Hereditary Haemochromatosis segregates with elevated erythrocyte mean corpuscular volume (MCV)

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Category:

Original research article – Brief communication

Key Words

HFE haemochromatosis, mean corpuscular volume, arthritis, C282Y, iron overload

Abstract

Objectives

To evaluate the relationship between erythrocyte parameters and the presence or absence of arthritis in HFE C282Y homozygous haemochromatosis (HH) subjects compared to control groups of non-HH subjects with arthritis.

Methods

Erythrocyte and arthritis parameters (mean cell volume, MCV and mean cell haemoglobin, MCH) were obtained from consecutive HH subjects (n=119) who were referred for initial evaluation and management. For comparison purposes, MCV and MCH values were collected from randomly selected non-HH subjects with rheumatoid arthritis (n=100) and osteoarthritis (n=100), consisting of equal numbers of men and women. Two other comparison groups comprised of 16 men and women who were heterozygous for C282Y with arthritis and 38 non-HH subjects with type 2 polyarticular osteoarthritis.

Results

MCV values were significantly higher in HH subjects with arthritis (95 ± 0.56 fL) than in HH subjects without arthritis (92.75 ± 0.50 fL, p=0.037). HH subjects with or without arthritis demonstrated a higher mean MCV compared to the control groups of non-HH osteoarthritis (90.12 ± 0.46 fL, p <0.001) and non-HH rheumatoid arthritis (90.94 ± 0.57 fL, p<0.001). HH subjects with arthritis also demonstrated a higher MCV when compared with heterozygous C282Y subjects with arthritis (93.18 ± 1.55 fL, p=0.025) and non-HH subjects with a similar pattern of arthritis, notably T2POA (91.13±0.50 fL, p<0.01). A MCV of 97.85 fL or greater provided a likelihood ratio of 2.2 for the development of arthritis in HH subjects.

Conclusion

This study has demonstrated a relationship between elevated MCV and arthritis in incident cases of HH.

1. Introduction

HFE haemochromatosis (HH) is an iron overload disorder affecting 1 in 200 individuals of northern European descent (1), usually caused by a homozygous C282Y mutation in the HFE gene (2,3). Up to 30% of individuals homozygous for C282Y may develop significant disease from iron overload, including HH-related arthropathy (4). HH-arthropathy was first described by Schumacher in 1964 and affects up to 81% of subjects (5). It is a significant cause of morbidity, disability and reduced quality of life (6). Classically, arthropathy affects the finger metacarpophalangeal (MCP) joints and other joints such as the hips, ankles, radio-carpal, elbow, shoulder and knee joints as well as the lumbar spine (7). It is unclear why arthropathy affects only a subset of people with HH, or whether it can be predicted.

Previous studies have reported that subjects with HH exhibit significant differences in peripheral blood erythrocyte parameters compared with controls (8,9). The mean cell volume (MCV) was elevated compared with healthy controls or those without HH, but with a range of joint diseases, predated the development of elevated body iron stores and persisted following therapeutic phlebotomy (9). Similarly, the arthritis of HH can also predate the development of iron overload and persist following phlebotomy therapy. Whether these HFE-related changes in erythrocyte parameters have predictive value in respect to arthritis in HH is unknown.

2. Aim

The aim of this study was to evaluate the relationship between erythrocyte parameters and the presence or absence of arthritis in HH subjects compared to randomly selected control groups of non-HH subjects with osteoarthritis and rheumatoid arthritis.

3. Methods

3.1 Participants

Between 2005 and 2015 consecutive HH subjects aged between 22 and 70 years with C282Y homozygosity who were referred for diagnosis, management and who underwent liver biopsy were included (n=119). Laboratory investigations included haematology, serum iron biochemistry, HH genotyping, liver biochemistry, chronic viral hepatitis serology, screens for autoimmune liver disease, Wilson disease and alpha-1 antitrypsin genotype. Hepatic iron concentration (HIC) was measured (10) and hepatic fibrosis graded (11). Exclusion criteria were: pregnant or lactating women, venesection in the previous 12 months, malignancy and acute inflammatory conditions. 66 of 119 HH subjects had arthritis (64 MCP 2,3 arthropathy, 20 both MCP and other joints). As characterization of arthropathy was not one of the routine clinical study objectives in the HH cohort, plain X-rays were not systematically undertaken. Phlebotomy treatment was performed weekly on all subjects until a serum ferritin level of less than 100µg/L was achieved, and the iron removed calculated as previously described (12). A second group of 16 men and women heterozygous for C282Y and in whom MCP 2,3 arthropathy with radiological features consistent with those observed in HH, but in whom there was no iron overload were included for comparison purposes. To evaluate differences with other chronic forms of arthritis, we randomly selected non-HH participants with rheumatoid arthritis (n=100) and osteoarthritis (n=100), consisting of equal numbers of men and women. Furthermore, a convenience sample of 38 participants who had clinically apparent type 2 polyarticular osteoarthritis involving the MCP 2,3 joints (T2POA) with radiological features consistent with those observed in HH, but in whom there was no iron overload and no detectable HFE gene mutations was also included for comparison purposes. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the QIMR Berghofer Medical Research Institute Human Research Ethics Committee.

3.2 Statistical analysis

All data are presented as mean ± SEM. Analysis of variance was used to determine significance of differences between groups using Brown-Forsyth and Welch's tests. Sensitivity and specificity analysis were performed using receiver operator characteristic curve analysis. Multivariate logistic regression analysis, including variables that were statistically significantly different in univariate analysis, was used to calculate the odds ratio (OR) for predictors of arthritis in subjects with HH. (Prism 8, GraphPad Software, CA). Statistical significance was assigned for $p<0.05$.

4. Results

Clinical characteristics of study subjects at initial diagnosis are shown in Table 1. MCV values were significantly higher in HH subjects exhibiting arthritis (95.00 ± 0.56 fL) versus HH subjects without arthritis (92.75 ± 0.50 fL, p=0.0375) (Figure 1), especially male HH subjects (95.33 ± 0.71 fL with arthritis versus 92.47 ± 0.72 fL without, p< 0.01). MCV values for the total combined group of all HH subjects with or without arthritis (93.87 ± 0.41 fL) and those with arthritis-only (95.00 ± 0.56 fL) were significantly higher when compared to the control groups of non-HH osteoarthritis (MCV 90.12 ± 0.46 fL, p <0.001) and non-HH rheumatoid arthritis (MCV 90.94 ± 0.57 fL, p<0.001) (Table 1). MCV values in the non-HH rheumatoid arthritis and osteoarthritis groups were similar. Subjects heterozygous for the C282Y mutation had significantly lower MCV values than HH subjects with arthritis (93.18 ± 1.55 vs 95 ± 0.56 fL, respectively, p=0.025), however there was no significant difference when compared with HH subjects without arthritis or non-HH subjects with osteoarthritis or rheumatoid arthritis. Sensitivity and specificity analyses demonstrated that a MCV of 97.85 fL or greater provided a likelihood ratio of 2.2 for the development of arthritis in HH subjects. No significant differences were observed for MCH in any of the cohorts.

Transferrin saturation was similar in HH subjects with or without arthritis (Table 1). Serum ferritin levels, HIC and iron removed were significantly higher in HH subjects with arthritis compared to those without (Table 1). Hepatic fibrosis was significantly greater in HH subjects with arthritis compared to those without (median Scheuer score 1 in those with arthritis versus 0 in those without fibrosis, $p<0.0001$, Mann Whitney test). Using linear regression analysis, no relationships were observed between MCV and either serum ferritin, HIC or iron removed. Multivariable logistic regression analysis confirmed fibrosis severity (OR 2.2, CI 1.1-4.7) and iron removal (OR 1.5, CI 1.3-2.3) exhibited ongoing association with arthritis.

5. Discussion

HH arthropathy is an aetiologic enigma and therapeutic challenge. Lack of insight into the mechanism responsible for joint disease in HH hinders the development of possible therapy and an inability to predict onset precludes the early implementation of possible corrective measures.

Our observation that HH arthropathy segregates with an elevated MCV needs to be verified in independent studies, but if confirmed, may permit earlier prediction of impending arthropathy and create opportunities for preventive and treatment strategies. Previous work shows that elevation of MCV precedes the elevation of serum ferritin levels in HH (9). Here we extend this observation by showing that MCV, iron burden, and liver fibrosis all associate HH-arthritis. Interestingly, the lack of a relationship between MCV and either HIC or iron removed suggests that the elevated MCV of HH is independent of increased iron burden per se. The MCV changes may be due to the direct effects of C282Y mutations in HFE per se. It is known that the HFE protein is not expressed in erythrocytes (13), with the elevated MCV in subjects with HH possibly being due to increased iron supply to erythroblasts (8). A

similar phenomenon could be occurring in cells that contribute to the genesis of arthritis. Thus, MCV may allow prediction of arthropathy propensity and allow scrutiny of those considered to be at greater risk. Indeed, our retrospective analysis demonstrated that a MCV of 97.85 fL or greater provided a 2.2-fold increased likelihood of development of arthritis in HH subjects.

Most information regarding disease pathogenesis in HH joints has been derived from surgical specimens obtained at joint replacement surgery, demonstrating iron deposition in joint synovium and cartilage (haemosiderin) (14, 15). Higher ferritin concentrations in synovial fluid, particularly prior to HH diagnosis and phlebotomy, may lead to greater uptake by synovial cells (fibroblasts and macrophages) and a substantial tissue deposition of iron, like the way urate accumulates until urate lowering therapy is used to treat gout. Likewise, chondrocytes may accumulate diffusible ferritin. Collectively these events, may lead to iron trapping and irreversible cellular iron accumulation. Over time, irreversible joint damage and painful arthropathy may ensue.

Strengths of this study include substantial numbers of participants, adequate numbers of controls including HFE heterozygous participants with similar arthropathy in whom both genders are well represented and the inclusion of both RA and OA control groups without HH. Weaknesses include the lack of radiographic evidence validating the arthropathies (all were physician diagnosed and supported by relevant laboratory data). No joint tissues were available to correlate findings.

Conclusion

In HH, elevated MCV segregates with arthropathy, unlike in osteoarthritis and rheumatoid arthritis. The mechanism underlying the erythrocyte manifestations is unclear. One possibility is that the increased erythrocyte MCV may be a function of dysregulated iron metabolism in

erythroblasts or abnormal intracellular iron processing in this cell lineage. A similar phenomenon may occur in joint tissues, which may in turn lead to iron-trapping and subsequent tissue damage. Whether perturbations of erythrocyte size may reflect iron deposition in joint tissues, including synovium, or iron retention in such tissues and so predispose to arthropathy, warrants further investigation.

Conflict of interest

The authors declare no conflict of interest.

Funding

No funding was received for this study.

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Figure legend

Figure 1. MCV in HH subjects with or without arthritis and control arthritis groups including osteoarthritis, rheumatoid arthritis, C282Y heterozygotes with MCP 2,3 arthritis and non-HFE gene mutation subjects with type 2 polyarticular osteoarthritis (T2POA) involving the MCP 2,3 joints (T2POA). Values are shown as the mean ± SEM. *$p<0.05$; **$p<0.01$; ***$p<0.001$ compared with the HH subjects with arthritis.

Table 1. Characteristics of HH subjects, with or without arthritis, osteoarthritis, rheumatoid arthritis, C282Y heterozygotes with arthritis and subjects with osteoarthritis resembling HH but without HFE mutations or iron overload.

	HH Subjects		Non-HH subjects			
	Arthritis	**No arthritis**	**Osteoarthritis**	**Rheumatoid arthritis**	**Heterozygotes**	**OA resembling HH arthropathy (T2POA)**
n (M/F)	66 (38/28)	53 (37/16)	100 (50/50)	100 (50/50)	16 (7/9)	38 (24/14)
Age ± SEM (yrs)	49 ± 2	40 ± 2	56 ± 2	62 ± 2	70 ± 2	74 ± 1.3
MCV ± SEM (fL)	95.00 ± 0.56	92.75 ± 0.50*	90.12 ± 0.46***	90.94 ± 0.57***	93.18 ± 1.55*	91.13 ± 0.45**
MCH ± SEM (pg)	32.38 ± 0.23	31.86 ± 0.20	30.12 ± 0.20	30.14 ± 0.23	31.30 ± 0.30	30.04 ± 0.30

TSat ± SEM (%)	85 ± 2	80 ± 2				
Ferritin ± SEM (μg/L)	1966±151	807±70 μg/L***				
HIC ± SEM (μmol/g)	237 ± 15	151 ± 10***				
Iron ± SEM removed (g)	11.6 ± 0.9	5.0 ± 0.4***				

HIC – hepatic iron concentration; MCH – mean cell haemoglobin; MCV – mean cell volume; OA – osteoarthritis; T2POA – type 2 polyarticular arthritis involving the MCP 2,3 joints; TSat – transferrin saturation. Iron parameters are only presented for the HH subjects. All statistical comparisons are with the HH arthritis group. *p<0.05; **p<0.01; ***p<0.001.