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ORIGINAL ARTICLE

Lack of association of iron metabolism and Dupuytren's disease

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Abstract

Background Iron accumulation as seen in genetic haemochromatosis is a major cause of hepatic fibrogenesis. A link between chronic liver disease and Dupuytren's disease (DD) is well established, especially in alcoholics.

Aim The aim of the present study was to test the hypothesis that iron accumulation might cause fibrosis of the palmar aponeurosis leading to DD.

Patients and methods We examined iron metabolism, mutations of the *HFE* gene, serum cholesterol, alcohol consumption, presence of chronic liver disease, diabetes and history of severe manual work in a group of 90 patients who had undergone surgery for a severe form of DD. The tissue removed during surgery was histologically examined to confirm the diagnosis of DD. For a control group, we used 33 healthy subjects with similar profiles.

Results The DD group consisted of 82 men and 8 women. Chronic liver disease was found in 27% of DD patients, compared with 6.1% of control subjects ($P = 0.013$). A history of hand traumatization was present in 33% of DD patients vs. 15% of control subjects ($P = 0.048$). Excessive alcohol consumption was present in 35.5% of DD patients compared with 15.1% of controls ($P = 0.029$). None of the other tested parameters, including the prevalence of *HFE* gene mutations, showed a significant difference between the two groups.

Conclusions Iron accumulation does not play a major role in the pathogenesis of DD. However, sex, age, manual labour and alcohol consumption are risk factors for progression of DD. We observed a high incidence of chronic liver disease in patients with DD.

Introduction

An excessive amount of hepatic iron leads to the deposition of collagen (i.e. hepatic fibrosis and eventually cirrhosis). This is particularly evident in genetic haemochromatosis (GH). As fibrogenesis is a common process, found in most types of tissues, an excessive amount of iron might contribute to the development of fibromatosis of palmar aponeuroses and eventually Dupuytren's contracture. This hypothesis is supported by the high incidence of both GH and Dupuytren's disease (DD) in populations of Nordic origin. Thus, we conducted the present study with the focus on the examination of iron metabolism in patients with DD.

Over the years, a causal relationship between iron accumulation in the liver and the development of hepatic cirrhosis has been demonstrated.[1,2] This is caused, mainly, by the high toxicity of ionized iron. Excessive free iron (as found in GH) is ionized with subsequent lipid peroxidation. This results in the hydrogenation of the double bonds in the unsaturated fatty acids that are constituents of membrane phospholipids. This hydrogenation reduces the integrity of the lipoprotein membranes of hepatocytes and their organelles, particularly the mitochondria.

The result is a derailment of intracellular homeostasis, leading to a decrease in adenosine triphosphosphate (ATP) concentration and, eventually, cell death. Hepatocyte necrosis activates the hepatic reticuloendothelial system

(RES) cells (Kupffer's cells) that release a variety of cytokines [e.g. transforming growth factor-beta (TGF-β) or tumour necrosis factor alpha (TNF-α)]. These substances initiate the transformation of hepatic stellate cells into myofibroblasts, which then produce large amounts of collagen, eventually leading to hepatic fibrosis.[1,2] A second, independent, pathway of hepatic fibrogenesis involving iron, is the direct effect of ionized iron on the stimulation of transcription of the gene that leads to pro-collagen formation in stellate cells.[1]

In many conditions with concomitant hepatic impairment due to other exogenous factors (viral infections, alcohol and other hepatotoxic xenobiotics), excess iron acts as a catalyst for pathological changes in the hepatic parenchyma and accelerates the development of hepatic cirrhosis.[1] Similar pathologic processes and morphologic changes are also seen in other affected tissues (pancreas, heart, endocrine organs).

DD is a condition characterized by palmar aponeurosis becoming hyperplastic with gradual retraction, most commonly of the 4th and 5th digits and culminating in a flexed fingers posture. The aetiology of the condition is not known. Current information on the pathogenesis of this disease suggests a relationship with hepatic fibrogenesis. The main histological feature is the hyperproduction of fibrous tissue, consisting principally of type III collagen, proteoglycans and fibronectin. These substances are produced by myofibroblasts. These cells (like activated hepatic stellate cells) produce collagen type III fibrils instead of, the normally prevalent, collagen type I. This is the basis for the mechanical changes that result in the clinical presentation of DD. The whole process takes place as a cascade, starting with the release of profibrotic cytokines (with TGF-β being the most important), which stimulates the transformation of fibroblasts into myofibroblasts. These myofibroblasts are capable of producing smooth muscle alpha actin and collagen type III.

Similar to hepatic fibrogenesis, stimulation of myofibroblasts is also related to free radicals. The possible significance of an excessive amount of iron related to the development of DD has not yet been studied.

Another interesting fact is that the highest prevalence of hereditary haemochromatosis in the world – similar to that of DD – is found in populations of north European descent.[2,3]

On the base of numerous clinical trials, the risk factors for DD have been classified into two basic groups:[4,1] Non-affectable factors, including genetics, sex and age, are well recognized and generally accepted as such and[2] affectable factors, including smoking, diabetes mellitus (DM), hypercholesterolemia, chronic trauma to the palmar aponeurosis, alcoholism, chronic liver disease and epilepsy. Although non-affectable factors are widely accepted, some studies[3,5] have cast doubt upon the role of affectable factors.

The present study was conducted to test our hypothesis that states that iron accumulation might be a contributing factor to the development of DD.

Patients and methods

Two groups of participating subjects were set up. Group A consisted of 90 unselected patients undergoing surgery for an advanced stage of DD with finger flexion due to palmar aponeurosis contracture in at least one hand. Inclusion criteria included disabling DD, absence of contraindications for surgery and Caucasian descent. All subjects were required to provide a written consent in order to participate in the study. Group A consisted of 82 men and 8 women, aged 29 to 81 years, with a mean age of 60.6 ± 9.7 years (mean ± standard deviation) and a mean body mass index (BMI) of 26.7 ± 3.5 kg/m^2. Group B (control group) included subjects of identical ethnic backgrounds, comparable age, sex ratio and BMI as those in Group A. Group B subjects were volunteers recruited from the city of Prague and the central Bohemia region of the Czech Republic. Prior to participation, the subjects underwent a routine physical examination by a GP or internist. Group B consisted of 33 subjects (30 men and 3 women, aged 26–89 years, with a mean age of 56 ± 16.3 years and a mean BMI of 25.9 ± 2.7 kg/m^2). Statistical comparisons between Groups A and B, relative to age and BMI, did not reveal any significant differences.

The assessment of the prevalence of C282Y and H63D mutations in the *HFE* gene in the control group was based on data about the prevalence of these mutations in the Czech Republic.[6,7]

The parameters assessed in both groups were as follows:

- Serum iron and ferritin levels, total iron binding capacity and transferrin saturation;
- Serum cholesterol levels;
- Presence of chronic liver disease (history, serum anti-HCV antibodies, Hepatitis B antigens, serum aminotransferase levels, ultrasound findings);
- Diabetes mellitus (history, fasting blood glucose levels);
- History of long-term strenuous manual work (> 5 years);
- Chronic alcohol abuse (> 60 g/day in men and > 20 g/day in women). The assessment was based on the patient's history. Alcohol users were asked about daily consumption of beer, wine and spirits. The quantities were recalculated to yield grams per day values. Values over 60 g per day in men and 20 g per day in women (i.e. amounts toxic to the liver parenchyma)[8] were considered significant.

Parameters assessed only in Group A were as follows:

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Table 1 Serum values of iron metabolism and cholesterol in the DD and control groups

Parameter	DD			Controls			
	Interval	Arithmetic mean	95% confidence interval	Interval	Arithmetic mean	95% confidence interval	*P*
Serum iron (μmo/L)	5.9–38	18.2	16.8–19.6	1.5–33	15.7	13.2–18.2	0.65 (NS)
Total iron binding capacity (μmo/L)	33–106	63.6	61.2–66.1	34–90	66.4	62.1–70.7	0.25 (NS)
Saturation of transferrin (%)	6.9–65.5	29.4	26.7–32.0	4.4–45.2	25.7	21.2–30.1	0.18 (NS)
Serum ferritin (ng/mL)	9.6–1118	223.4	179.4–267.4	46.4–666	217.1	136.1–298.1	0.89 (NS)
Serum cholesterol (mmol/L)	2.4–9.0	5.7	5.4–5.9	2.3–7.7	5.4	4.9–5.8	0.25 (NS)

NS, not significant.

- Histological examination of palmar aponeurosis tissue specimens stained with haematoxylin-eosin and Perls methods to confirm diagnosis of DD and to assess iron deposition;
- Examination of the *HFE* gene for the presence of the two most frequent mutations (C282Y and H63D) using the PCR-RFLP method as described in a previous study.[7] Briefly, DNA was extracted from the blood samples by QiaAmp DNA Mini Kit spin columns (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Initially, Feder's primers for amplification of the regions containing the C282Y and H63D mutations were used;[9] later, new primers for C282Y mutation were designed: forward primer, 20-mer 5′-AACCTTGGCTGTACCCCCTG-3′ and reverse primer, 20-mer 5′-GCCCACCCCCTAACAAAGAG-3′.[7] Digestion was done with *Rsa*I and *Bcl*I for C282Y and H63D mutations, respectively, as described previously.[7,9] Resulting fragments were visualized using ethidium bromide staining following gel electrophoresis.

  The study was approved by the Ethics Committee of the Faculty Hospital Kralovske Vinohrady.

Table 2 Prevalence of H63D and C282Y mutations in the DD and control group (80 DD patients were examined)

Mutation	DD		Controls		*P*
	n	%	*n*	%	
C282Y Ht	5/80	6.2	33/481	6.9	0.84 (NS)
C282YHm	0/80	0	0/481	0	–
H63D Ht	25/80	31.2	128/481	26.6	0.38 (NS)
H63D Hm	1/80	1.2	8/481	1.7	0.78 (NS)

Ht, heterozygotes; Hm, homozygotes.

Table 3 Prevalence of risk factors in the DD and control groups

Risk factor	DD		Controls		*P*
	n	%	*n*	%	
Chronic liver disease	24/90	26.7	2/33	6.1	0.013
Diabetes	24/90	26.7	6/33	18.2	0.336 (NS)
Manual work	30/90	33.3	5/33	15.1	0.048
Alcohol abuse	32/90	35.5	5/33	15.1	0.029

Statistical analysis

The data were statistically analysed using the Student's *t*-test and the Fisher's exact test using the SigmaStat program (Jandel Scinetific, USA). The design of the study and statistical results were reviewed by a statistician.

Results

- The differences between serum iron values, total iron binding capacity, saturation of transferrin, serum ferritin and cholesterol concentrations between groups A and B were not statistically significant (Table 1).
- The prevalence of HFE gene mutations C282Y and H63D in groups A and B was not significantly different (Table 2).
- In group A, 27% (24 of 90) patients were diagnosed as having chronic liver disease [aetiology: alcohol, 75% (18 of 24); type C chronic hepatitis, 4.2% (1 of 24); non-alcoholic steatohepatitis, 4.2% (1 of 24); primary biliary cirrhosis, 4.2% (1 of 24); and idiopathic, 12.5% (3 of 24)]. In group B, only 6.1% (2 of 33) had chronic liver disease. This difference was statistically significant. Similarly, chronic hand traumatization and the percentage of alcohol abusers were significantly higher in group A. The prevalence of diabetes did not differ significantly between the two groups (Table 3).

All tissue samples of the palmar aponeuroses (cords and/or nodules) in Group A were histologically diagnosed as DD. We did not differentiate between nodules and

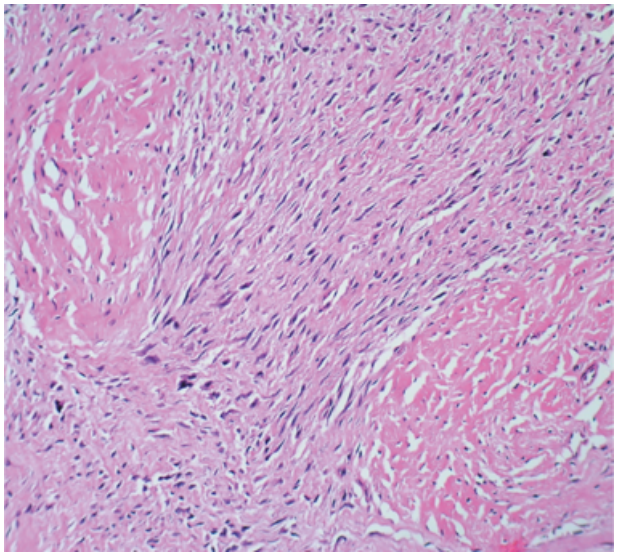

fig. 1 Palmar fibromatosis (Dupuytren's contracture) with no iron storage. Haematoxylin-eosin stain. Original magnification ×20. Author: Stritesky.

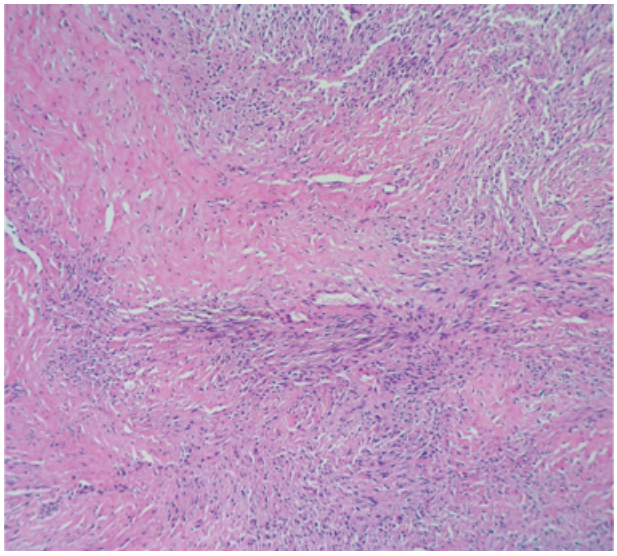

fig. 2 Iron storage in palmar fibromatosis (Dupuytren's contracture). Haematoxylin-eosin stain. Original magnification ×10. Author: Stritesky.

cords; a histologic picture of palmar fibromatosis was the main diagnostic criterion (fig. 1). Samples from two subjects (2.2%) showed small amounts of iron-laden macrophages upon staining with Perls stain (figs 2 and 3).

Discussion

The aetiology of DD is not known. DD is considered to be an autosomally dominant hereditary condition. No single DD gene has been found, so far, and it is assumed that the development of DD is based on complex interactions of various genes.[10] Moreover, gene penetration is fairly variable. Thus, manifestation of the disease is significantly influenced by the external environment that may provide triggering mechanisms. These mechanisms are varied and numerous. They include microtraumatization of the tissue resulting in hypertrophic inflammation, various kinds of microangiopathies, which lead to microcirculation disorders and tissue hypoxia, as well as metabolic derangement. The common consequence of these effects is the development of inflammatory changes accompanied by the production of cytokines, particularly TGF-β.

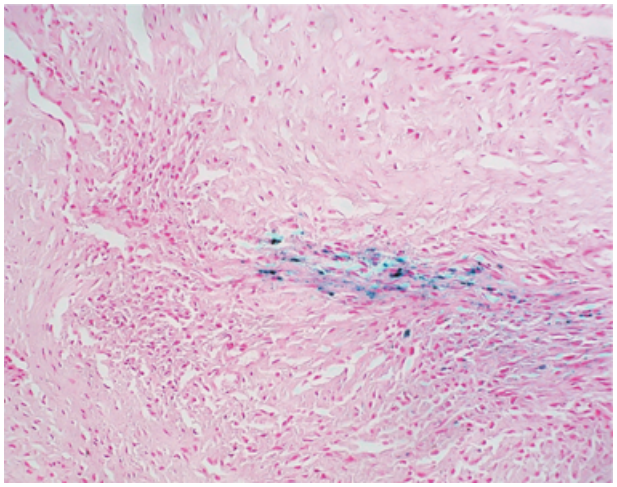

fig. 3 Iron storage in palmar fibromatosis (Dupuytren's contracture). Perls stain. Original magnification ×20. Author: Stritesky.

Our study did not confirm the presumed effect of iron accumulation on the development of DD. No increase in prevalence of the GH genotype was observed among DD patients. Similarly, iron staining of the surgically removed palmar aponeuroses did not reveal iron accumulation. Thus, the role of iron in the pathogenesis of DD is trivial, at best. This might be related to the generally low accumulation of iron in tendons and aponeuroses. Moreover, some tissues seem to be protected against iron toxicity even in the face of excessive amounts in the organism.

Age, sex, alcohol abuse and strenuous manual work were statistically important risk factors for the development of DD. This corroborates the results of recent studies dealing with similar topics.[3,11,12] Rather surprisingly, we did not find a higher prevalence of diabetic patients and hypercholesterolemia in the DD group; however, this is consistent with findings in the Reykjavik study.[13] A high prevalence of diabetics in our control group (18%, 6 of 32) reflects the prevalence of diabetes in the Czech population over 60 years of age.[14] Other frequently mentioned risk factors for the development of DD include genetic

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predisposition, nicotine and epilepsy; however, these factors were not examined in our study.

Apart from surgery, no effective treatment for advanced DD is known. However, elimination of affectable risk factors is believed to reduce the disease prevalence or to meliorate its course.

Conclusions

Iron metabolism in patients undergoing surgery for advanced forms of DD did not differ significantly from the control group. In addition, histological examination of palmar aponeuroses from patients with DD did not reveal any significant iron accumulation. Compared with the general population, no significant difference in the prevalence of *HFE* gene mutations C282Y and H63D were seen in DD patients. Relative to the other parameters studied, the DD group did not show an increased incidence of hypercholesterolemia or diabetes.

On the other hand, the DD group had a higher prevalence of elderly men, chronic liver disease, alcohol abusers, as well as, subjects with a history of strenuous manual work. According to our findings, these are identifiable risk factors for the development of DD.

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