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Hereditary hemochromatosis associated with the development of liver cirrhosis

Hemocromatose hereditária associada ao desenvolvimento da cirrose hepática

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ABSTRACT

Hereditary hemochromatosis (HH) is an autosomal recessive disease, most often associated with mutations in the *HFE* gene, which result in continuous absorption of iron, causing its overload. Liver tissue is the main site of iron deposition; thus, high levels of iron, when interacting with oxygen, induce the formation of free radicals that will act on proteins, lipids, and deoxyribonucleic acid (DNA), which may trigger deleterious effects at cellular and tissue levels. In order to elucidate the development and progression of liver cirrhosis due to iron overload, the purpose of this study is to describe the pathophysiology of the hepatic system in patients diagnosed with HH. For this purpose, searches for scientific articles were carried out in the main academic databases. We found that patients diagnosed with HH are more likely to develop liver cirrhosis, since chronic iron deposition in liver tissue induces injury and consequent tissue regeneration, progressing to collagen fibers synthesis surrounding the hepatocytes, leading to loss of liver function and development of cirrhosis. Therefore, it is necessary to carry out tests such as iron, ferritin and transferrin measurements, to evaluate body's iron stores, aiming at an early diagnosis of iron overload, thus avoiding deleterious damage at cellular and tissue levels.

Key words: iron metabolism disorders; mutation of the *HFE* gene; hereditary hemochromatosis; liver cirrhosis.

RESUMO

A hemocromatose hereditária (HH) é uma doença autossômica recessiva, associada, na maioria das vezes, a mutações do gene HFE*, que resultam em absorção contínua de ferro, ocasionando a sobrecarga dessa substância. O tecido hepático é o principal sítio de depósito do ferro; dessa forma, níveis elevados de ferro, ao interagir com o oxigênio, induzem a formação de radicais livres que irão agir sobre proteínas, lipídios e ácido desoxirribonucleico (DNA), podendo desencadear efeitos deletérios a níveis celulares e teciduais. Visando elucidar o mecanismo de desenvolvimento da cirrose hepática decorrente da sobrecarga de ferro, o objetivo deste estudo é descrever a fisiopatologia do sistema hepático em pacientes diagnosticados com HH. Para isso, foram realizadas buscas por artigos científicos nos principais bancos de dados acadêmicos. Verificamos que pacientes diagnosticados com HH apresentam maior predisposição de desenvolver cirrose hepática, pois o depósito crônico de ferro no tecido hepático provoca lesão e consequente regeneração tecidual, progredindo para formação de fibras de colágeno que circundam os hepatócitos, levando à perda da função hepática e ao desenvolvimento da cirrose. Diante disso, faz-se necessária a realização de exames como dosagem de ferro, ferritina e transferrina para avaliação dos estoques de ferro do organismo, objetivando um diagnóstico precoce da sobrecarga de ferro, a fim de evitar danos deletérios a níveis celulares e teciduais.*

Unitermos: *desordem do metabolismo do ferro; mutações do gene* HFE*; hemocromatose hereditária; cirrose hepática.*

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RESUMEN

La hemocromatosis hereditária (HH) es uma enfermedad autosómica recesiva, asociada, la mayoría de las veces, a mutaciones del gen HFE*, que producen absorción continua de hierro, con sobrecarga de esa sustancia. El tejido hepático es el principal sitio de almacenamiento de hierro; así, niveles elevados de hierro, al interactuar con oxígeno, inducen la formación de radicales libres que actuarán sobre proteínas, lípidos y ácido desoxirribonucléico (ADN), pudiendo acarrear efectos dañosos a nível celular y tisular. Para aclarar el mecanismo de desarrollo de la cirrosis hepática debido a sobrecarga de hierro, el objetivo de este estudio es describir la fisiopatologia del sistema hepático en pacientes diagnosticados con HH. Para eso, se efectuaron búsquedas por artículos científicos en los principales bancos de datos acadêmicos. Verificamos que pacientes diagnosticados con HH presentan mayor predisposición a desarrollar cirrosis hepática, porque el depósito crônico de hierro en el tejido hepático causa lesión y consecuente regeneración de tejido, progresando a la formación de fibras de colágeno que rodean los hepatocitos, llevando la pérdida de la función hepática y al desarrollo de la cirrosis. Ante esto, es necesario medir hierro, ferritina y transferrina para evaluación de las provisiones de hierro del cuerpo, buscando un diagnóstico temprano de la sobrecarga de hierro, para evitar efectos deletereos a nível celular y tisular.*

Palabras clave: *trastornos del metabolismo del hierro; mutaciones del gen* HFE*; hemocromatosis hereditaria; cirrosis hepática.*

INTRODUCTION

Iron is an essential mineral for the organism due to its ability to donate and receive electrons, participating in several biological reactions. It plays a vital role in the transport of oxygen and cellular energy production, and is a major component in synthesis of heme molecule, present in hemoglobin, in addition to participating in the synthesis of several proteins[1, 2].

Unlike other metals, iron is highly stored by the body. Thus, blood and tissue iron levels must be maintained in concentrations appropriate for their use. When levels are low or high, a regulated sequence of protein synthesis is triggered to ensure the recovery of iron levels in the body[1, 2].

Typically, high concentrations of iron in plasma induce the activation of command sites, which will reduce their levels through two mechanisms, one intracellular, according to the amount of iron present in the cell, and another systemic, in which hepcidin has a key role. Hepcidin is a 25 amino acids, disulfide-rich peptide, with hormonal action, in which the gene is located on chromosome 6; it is synthesized and secreted by several cells, and the liver tissue is its main production site[3].

Iron overload results mainly from changes in the synthesis of this protein. In general, these changes will result in increased iron absorption and the consequent increased iron serum levels, since this protein is responsible for the final adjustment of the systemic regulation of iron homeostasis[3].

Hemochromatosis is the main disease related to this disorder. It can be classified as of primary origin – hereditary hemochromatosis (HH) –, genetic disease – associated, in most cases, with mutations in the *HFE* gene, of which C282Y and H63D are the most frequent –, or of secondary origin, which is related to other pre-existing conditions or environmental factors[4, 5].

HH is defined as an autosomal systemic disease, resulting from changes in protein genes involved in iron regulation. After the discovery of the *HFE* gene mutation in 1996, it is now classified as one of the most frequent genetic diseases in humans, mainly in the Caucasian population[4, 5]. It is characterized by an increase in the intestinal absorption of iron, which results in its overload, and thus, may trigger deleterious effects at cellular and tissue levels with the consequent development of diseases[6].

Among the main complications of HH are liver cirrhosis (LC) and hepatocellular carcinoma (HCC)[7]. The process to trigger a cirrhotic condition is slow and requires the tissue to be exposed to a high concentration of iron for long periods. When this occurs, the liver tissue undergoes an inflammation and tissue repair process. This repair will trigger fibrosis, which is characterized by the develpment of connective tissue that replaces hepatocytes in an attempt to regenerate and repair tissue damage, thus blocking blood circulation; this causes the hepatic tissue to decrease its elasticity and occurs loss of liver system function[8, 9].

There is a lack of articles in the literature directed to the study of LC resulting from HH. However, it is believed that iron overload in a progressive manner is extremely harmful to hepatocytes, due to their participation in the activation of stellate cells with the consequent development of collagen fibers, which convert the normal structure of the liver, triggering cirrhosis. In this context, there is a need to conduct studies aiming to elucidate

the pathophysiology of HH, emphasizing their relationship in the development of LC.

OBJECTIVES

General objective

To discuss, based on data in the literature, the relationship between HH and LC, clarifying how patients diagnosed with this metabolic disorder develop this pathological condition.

Specific objectives

• To describe the iron metabolism and the pathophysiology of the hepatic system in HH;

• to present the risk factors associated with the development of HH;

• to list the conditions associated with the development of LC in HH.

MATERIALS AND METHODS

It is a literature review with a narrative approach, thus, aiming to synthesize the current knowledge on the theme addressed, in order to identify and analyze results of studies on the same subject. We analyzed, using data available in publications, whether patients diagnosed with HH are more likely to develop LC.

Data collection was conducted by reading scientific articles published both in Portuguese and in English, in the Scientific Electronic Library Online (Scielo) and the National Library of Medicine – NIH (PubMed) databases, using descriptors such as: *desordem do metabolismo do ferro*; iron metabolism disorders; *mutações do gene* HFE; *HFE* gene mutations; *hemocromatose hereditária*; hereditary hemochromatosis; *cirrose hepática*; liver cirrhosis. Boolean expressions AND and OR were used to find records in which the descriptors were combined. Another strategy used was the manual search of the reference lists of the identified and selected articles, in addition to the American Association for the Study of Liver Diseases (AASLD) publications. The search in the databases provided 1508 articles in PubMed and 10 in Scielo, totaling1518 articles. From these, 844 articles were excluded because they were not within the stated period (1995-2019), due to the languages proposed by the review, and for not including the abstract available. The remaining 674 were subjected to title and key words reading; from this total, 445 did not fit the theme and the issue of the review. After reading 229 articles summary, 109 articles were selected for complete and critical reading. After reading those in full, 30 – dating 1995-2018 – were selected to be part of this narrative literature review, as described in the flowchart below (**Figure 1**).

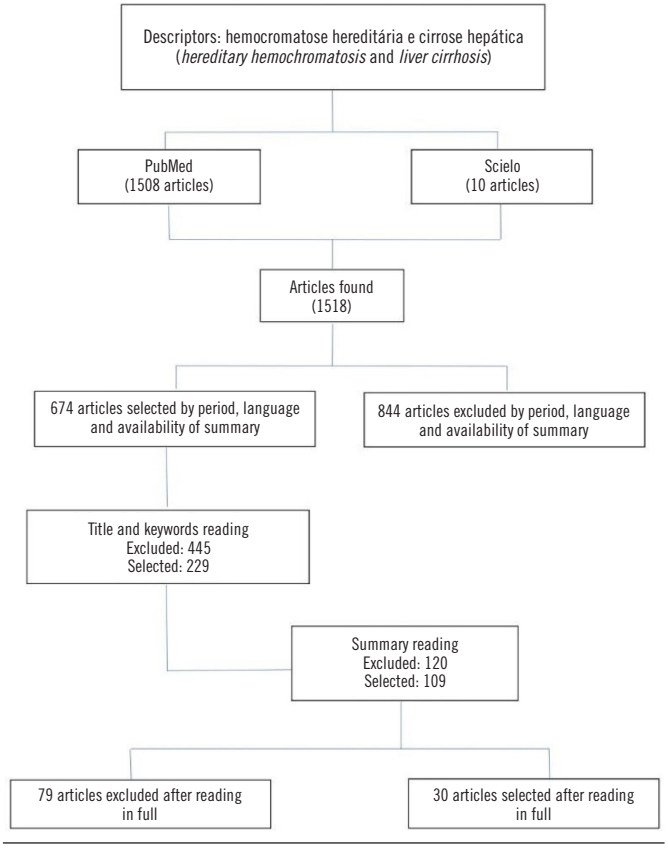

FIGURE 1 – *Flowchart of articles inclusion and exclusion process*

Theoretical reference

Iron metabolism

Iron is an essential inorganic ion for the organism due to its ability to donate and receive electrons, participating in several biological processes. Unlike other metals, iron is highly conserved by the body; therefore, the role of the proteins involved in its metabolism is essential for the stability of this mineral. When blood and tissue iron levels are decreased or elevated, a regulated sequence is triggered by enzymatic and degradation mechanisms to ensure the restoration of its normal levels in the body[1, 2].

The human body has two main sources of iron: from the diet and the recycling of senescent erythrocytes. In the diet, about 1-2 mg of iron is absorbed in its inorganic form or heme form, by the duodenal epithelium, to guarantee the metabolic balance

according to the body needs. When recycling erythrocytes, the daily reuse is approximately 0.8% (release 20 mg of iron), and reused in the bone marrow to produce new erythrocytes (RBCs)[1].

Iron homeostasis is regulated by two mechanisms, one intracellular, according to the amount of iron present in the cell, and another systemic, via hepcidin, a peptide chain of 25 amino acids, with hormonal action, whose gene is located on the chromosome 6. This molecule can be synthesized and secreted by several cells, and the liver tissue is its main production site. The deficiency of this protein induces iron overload, while its excess induces anemia due to deficiency of this mineral[1, 10].

Usually, iron is eliminated from the body by bodily secretions, sloughing of intestinal cells and skin peeling, or menstrual bleeding. Under iron homeostasis, excretion mechanisms are less developed and effective when compared to absorption and regulatory mechanisms. Thus, the control of iron levels requires communication between the absorption, utilization and storage locations, mediated by hepcidin[3, 11].

Absorption, uptake and regulation

Dietary iron can be found in ferrous and ferric iron form, the former is better absorbed than the latter. Iron absorption is mediated by the upper duodenal epithelium, which consists of villous structures to increase the surface area for absorption. Factors such as acidity and the presence of solubilizing agents favor intestinal absorption[1, 3, 12].

Ferric iron (Fe^{+3}) from the diet is absorbed by enterocyte and converted into a ferrous state (Fe^{+2}), mediated by the duodenal ferric reductase enzyme (duodenal cytochrome B – Dcytb) and the six-transmembrane epithelial antigen of prostate 3 (STEAP3) protein [3]. In contrast, the absorption of Fe^{+2} is less determined. Evidently, internalization occurs by the heme carrier protein 1 (HCP1), at the apical edge of the duodenal cell[3].

After absorption, Fe^{+2} is transported into the cytoplasm by the divalent metal transporter 1 (DMT1), which can be used by the cell, restrained as ferritin or released from the enterocyte into the plasma, depending on the demand for iron. If the requirement is low, it will remain in the enterocyte sequestered by ferritin and will be eliminated when intestinal epithelium sloughing occurs. If there is a need, it will be transported outside the enterocyte towards the plasma[1, 3].

Ferroportin (FPN) is a molecule present in the basolateral end of cells of the reticuloendothelial system (RES) and in enterocytes; it plays an important role in export cellular iron to plasma, and is the only mechanism of iron flow. As well as DMT1, it is also selective for iron in the form of Fe^{+2}[13]. FPN, in addition to exporting cellular iron, is also the recipient of hepcidin, which regulates the function of FPN, inhibiting the export of iron; thus, in the event of higher concentrations of hepcidin in the plasma, most of the iron absorbed will be retained as ferritin in the enterocyte[13-15].

Hephaestin, a transmembrane oxidase enzyme, acts on the conversion of Fe^{+2} to Fe^{+3}. It prepares Fe^{+3} to be captured and transported by transferrin (Tf), a glycoprotein synthesized and secreted by the liver – the main iron transport protein[16, 17].

Transferrin-bound iron is taken up by cells through interaction with transferrin receptor 1 (*TfR1*); as a result, it is reduced by the STEAP3 ferric reductase, which, in turn, acts as a facilitator of the dissociation of the iron-Tf complex and as a Fe^{+3} carrier through the endosomal membrane to the cytoplasm by DMT1[16, 18, 19]. Another Tf receptor (*TfR2*) is expressed on the hepatocyte membrane and binds to the iron-Tf complex with a lower degree of affinity when compared to the *HFE-TfR1* interaction[20-22].

In normal conditions, almost all non-heme iron in circulation is bounded to the Tf molecule, due to its affinity for iron at the physiologic pH. However, in events of iron excess, when the binding capacity of Tf is saturated, non Tf bound iron (NTBI) appears in the plasma[3, 16].

The increase in plasma iron concentration physiologically induces the signaling of command sites to reduce their circulating levels, thus protecting the organism to prevent toxicity associated with iron overload by hepcidin production. When plasma iron is reduced, signaling for hepcidin synthesis is disrrupted and its levels decrease[14, 23].

Hepcidin, a circulating peptide hormone that plays a negative regulatory role in iron metabolism, is encoded by the hepcidin antimicrobial peptide gene (*HAMP*). It is synthesized primarily by hepatocytes and subsequently processed and secreted into the circulation[24].

The *HAMP* gene is responsible for hepcidin transcription. Its expression is regulated by iron levels, so that iron overload increases its expression. The transcription of the *HAMP* gene is related to the action of the *HFE*, *TfR2*, and hemojuvelin (HJV) proteins. Evidently, HJV is a coreceptor of bone morphogenic proteins (BMPs), cytokines with important functions in tissue regulation of proliferation, differentiation, apoptosis, and migration. The excessive intra-hepatocyte iron raises the BMPs expression, which in a paracrine fashion bind to HJV. Subsequently, activation of the intracellular signaling cascade of SMAD proteins that induce increased hepcidin synthesis occurs[11, 18, 25, 26].

Plasma iron overload leads to *HFE* activation, expressed in the hepatocyte membrane through interaction with β2-microglobulin (β2M). Once activated, the *HFE* protein binds to the Tf receptor 1, recognizing the circulating iron-Tf complex. *HFE* is disassociated from *TfR1* and bound to *TfR2*, thereby activating hepcidin transcription by signaling via ERK or BMP/SMAD[22, 26]. Hepcidin, regulated by *HFE*, degrades the iron transporter (FPN) in the cytoplasmic membrane of enterocytes and macrophages, resulting in decreased iron uptake and extracellular supply[11, 27].

Changes in one of the protein synthesis pathways involved in iron homeostasis that affect the appropriate hepcidin expression, such as inflammation, ineffective erythropoiesis or hypoxia, cause increased iron absorption and the consequent increase in serum irom levels[3].

Iron overload

Iron overload can be classified as hereditary or secondary. Hereditary iron overload is the result of mutations in protein genes related to iron homeostasis in the body. This metabolic disorder is observed in patients with HH who have an inappropriate increase in the intestinal absorption of the mineral, most often associated with the mutation of the *HFE* gene[6]. Secondary iron overload is associated with underlying causes, which result in interference from iron regulatory pathways, or directly linked to the increase in its storage, such as hemolytic anemia and/or ineffective erythropoiesis that require multiple blood transfusions[28].

The severity of iron overload is related to the specific importance of the compromised regulatory protein. In conditions where there is a loss of one of the iron regulatory proteins (IRP) involved in iron homeostasis, there is no effective control of iron absorption by the enterocyte, resulting in its continuous release into the plasma and consequent gradual accumulation of iron in parenchymal tissue. While Tf is saturated, the NTBI concentration increases. Therefore, when free iron reacts with oxygen it induces the development of free radicals, which will act on proteins, lipids and deoxyribonucleic acid (DNA) that may trigger deleterious effects at cellular and tissue levels, favoring the development of diseases[3, 16, 29].

HH

HH is defined as a systemic, autosomal recessive disorder, resulting from mutations in proteins genes associated with iron regulation that cause elevation of Tf saturation, causing the progressive accumulation of iron, which is deposited and stored in several tissues, notably in the pancreas, in the heart, in the pituitary and, to a greater extent, in the liver parenchyma[6, 30, 31].

Five types of HH were identified according to clinical, biochemical and genetic characteristics; from a histological point of view, they are very similar due to a common pathophysiology that involves hepcidin production, whose levels modulates the severity of iron load[15] (**Table**).

TABLE – Genes related to HH

HFE-related HH
Non-*HFE* hemochromatosis
• Juvenile hemochromatosis
(subtypes: 2A associated with *HJV* gene and 2B associated with *HAMP* gene)
• *TfR2*-related hematochromatosis
• Ferroportin disease associated with *SLC40A1* gene

Source: Adapted from Pietragelo (2004)[15].

HH: hereditary hemochromatosis; HFE*: HH gene; HJV: hemojuvelin;* HAMP*: hepcidin antimicrobial peptide;* TfR2*: Transferrin receptor 2;* SLC40A1*: ferroportin gene.*

HFE-*related HH*

Type 1 hemochromatosis is the most prevalent of HHs. It is an autosomal recessive disease that is associated with iron overload with consequent dysfunction of several organs[4, 6].

In 1996, Feder *et al*. (1996)[32] identified the hemochromatosis gene, called *HFE*, in the short arm of chromosome 6. Mutations that induce the substitution of cystine for tyrosine amino acid at position 282 (C282Y/C282Y) in homozygosis are identified in 85% to 90% of cases of typical HH, in patients of northern European origin. A second mutation, resulting from the substitution of histidine for aspartate, was identified at position 63 (H63D). This allele was identified as a cause of HH when associated with C282Y (C282Y/H63D) and rarely when homozygous, which may increase hepatic iron levels; however, it does not result in iron overload[4, 5, 31].

Non-HFE *HH*

Non-*HFE*-related HH is a term used to define hemochromatoses that are not associated with mutations in the *HFE* gene. It includes HH types 2A, 2B, 3 and 4. These are rare diseases, and are considered when HH cannot be explained by mutations in the *HFE* gene[15].

Juvenile hemochromatosis (JH) type 2 is a rare disorder, with autosomal recessive inheritance, in which iron levels are high after the second decade of life. It is divided into two subtypes: JH type 2A, caused by mutations of the *HJV* gene located on chromosome 1q21, and type 2B JH, caused by mutations of the *HAMP* gene, positioned on chromosome 19q13[25, 33].

Hemochromatosis type 3 is a mutation of the *TfR2* gene located on chromosome 7q22, with a autosomal-recessive inheritance; it is regarded as HH *HFE* in terms of changes in laboratory parameters, complications and consequent hepatic iron storage[22].

Hemochromatosis type 4, known as FPN disease, is caused by mutations in the *SLC40A1* gene, in which hepcidin levels are normal. However, FPN does not respond to hepcidin stimuli, thus exacerbating the release of iron into the circulation, leading to saturation of the blood compartment of iron[15].

Hepatic system

The liver is a subdiaphragmatic glandular organ, structurally divided into lobes, organized around the hepatic vein with the portal triads located at its periphery. In the normal liver, interstitial collagen types I and III, are concentrated in the portal tracts and around the central veins[34, 35].

Hepatocytes are arranged around the hepatic lobules, forming cellular plaques, which are directed from the periphery of the lobe to its center. Hepatocyte plaques are flanked by vascular sinusoids – they are irregularly dilated vessels, composed of fenestrated discontinuous layer of endothelial cells –, which contain a low-density matrix of basement membrane marked by the space of Disse, where resident macrophages, lymphocytes, mostly natural killer (NK) cells, Kupffer cells, liver type hepatic stellate cells (HSC) and type IV collagen fibers. In an inactive state, HSC are responsible for the storage of lipid substances and vitamins; in an active state, they are the main fibrogenic cells of the liver tissue[36, 37].

RESULTS AND DISCUSSION

Metabolic changes in HH – participation of the *HFE* gene in the regulation of intestinal iron absorption

In 1998, Zhou *et al*. (1998)[38] found that individuals homozygous for C282Y mutation had less interaction between *HFE* and its light chain β2M. The study conducted by Santos *et al*. (1996)[39] demonstrated that mice with β2M mutation are unable to limit the transfer of iron from mucosal cells to plasma, showing abnormally high Tf saturation.

Nicolas *et al*. (2004)[40] reported in a study that changes in the hepcidin expression may contribute to phenotypic variability in clinical expression and abnormalities of iron homeostasis in human patients with HH. A similar conclusion was reached by Merryweather-Clarke *et al*. (2003)[41], who identified mutations of the que *HAMP* gene, responsible for expressing hepcidin in two families with HH. They observed a correlation between the severity of iron overload and the presence of mutations of hepcidin in individuals homozygous for *HFE* C282Y mutations.

In this situation, C282Y/C282Y mutation causes decreased of the *HFE* gene expression and reduced affinity and expression of β2M, resulting in increased iron absorption due to blockage in hepcidin production and enterocyte flow into the circulation, with consequent increased Tf saturation, leading to hemochromatosis[39, 42, 43].

Pathophysiology of LC development due to chronic iron deposition in patients diagnosed with HH

Studies report that most C282Y homozygotes will have evidence of increased iron stores[44]. Population screening surveys show that between 75% and 94% of homozygotes will have high Tf saturation, and 64% to 68% will have increased serum ferritin level[31, 44-46]. In all studies, men were shown to be more susceptible than women.

Wood *et al*. (2008)[47] showed, through meta-analysis, that among the individuals genetically predisposed to the homozygous C282Y mutation, 75% to 100% of men will have biochemical evidence of increased iron storage, and 10% to 25% of men may present fibrosis on biopsy, while 4% to 6% will have mild cirrhosis. The ratio of women affected was shown to be much lower.

Pathophysiology of cell damage and liver fibrosis related to iron overload

Cellular damage due to iron overload deposited in liver tissue occurs when the plasma amount of iron exceeds the Tf saturation capacity. Free iron acts as a catalyst for oxidative reactions, with the consequent generation of reactive oxygen species (ROS), which promote the lipid peroxidation of the membrane of several cytoplasmic organelles, leading to cell damage, fibrosis and functional failure[48, 49].

In a study, Arezzini *et al*. (2003)[50] demonstrated that mice receiving an iron-rich diet had high collagen deposition in hepatocytes; it was also possible to observe mild fibrosis in a given population, as well as evidence of LC in those exposed to higher amounts of iron.

Liver fibrogenesis, a normal tissue repair process, is mediated by a complex regulated signaling network between hepatocytes, HSC, sinusoidal endothelial cells, Kupffer cells, biliary epithelial cells, lymphocytes associated with liver and non-resident infiltrated cells[51]. In response to hepatocyte damage and dysfunction and increased oxidative stress, stellate cells are activated, which, due to tissue damage are stimulated to secrete several profibrogenic

cytokines, such as platelet-derived growth factor receptor-β (PDGFR-β). Concurrently, Kupffer cells and lymphocytes release cytokines and chemokines, such as the transforming growth factor β (TGF-β). The increase in these cytokines modulates the expression of genes in hepatic stellate cells, which differentiate into myofibroblasts, with subsequent proliferation and greater collagen synthesis (types I and II), and production of cell matrix components, which are deposited in the space of Disse and are involved in fibrogenesis, characterizing the gradual development of fibrosis (**Figure 2**)[51-53].

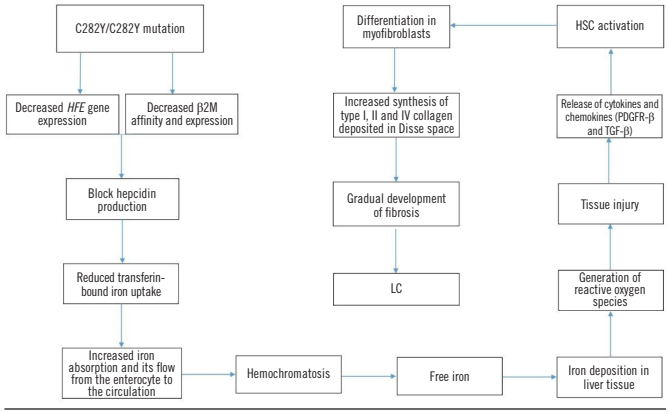

FIGURE 2 – *Flowchart of the pathogenesis of LC from HH*

LC: liver cirrhosis; HH: hereditary hemochromatosis; β2M: β2-microglobulin; HSC: hepatic stellate cells; PDGFR-β: platelet-derived growth factor receptor beta; TGF-β: transforming growth factor beta.

In 2003, Nicolas *et al*. (2003)[14] reported an increase in collagen deposition in the portal tracts of mice after simultaneous disruption of *HFE* and another important regulator of iron homeostasis, the *TfR2* receptor. In addition, they described the development of liver fibrosis in mice with *HAMP* mutation, fed with an iron-rich diet.

In 1995, Stal *et al*. (1995)[53] studied signs of Kupffer cell activation and inflammatory responses in liver biopsy samples collected from 15 patients with untreated hemochromatosis and six patients with treated hemochromatosis. Using immunohistochemistry, they observed that three of the untreated patients (20%) had cirrhosis, and eight (53%) had fibrosis.

Liver fibrosis can be classified in several stages according to the progression, and the regeneration of hepatocytes is stimulated and subsequent scarring that aggregate to each other in the liver tissue. These scars, formed by the accumulation of collagen and components of cellular matrix, are able to compress the sinusoidal channels and increase vascular resistance in the liver parenchyma, since they form fibrotic bridges that carve the liver into regenerative nodules. When the liver lobules are completely surrounded by scars, presenting a micronodular pattern, liver cirrhosis can be diagnosed[54, 55].

LC – continuous pathogenic consequence of the long clinical course of chronic iron deposition in the tissue

Valenti *et al*. (2010)[56] demonstrated that iron accumulation predominantly in hepatocytes is associated with a risk 1.7 times greater in the development of a fibrosis stage higher than 1, in comparison with the absence of siderosis.

The data presented by Allen *et al*. (2008)[44] showed liver fibrosis or cirrhosis in 7% of their general cohort of 158 C282Y homozygotes. Olynyk *et al*. (2005)[49] evaluated the results of 60 liver biopsies from individuals diagnosed with HH; of which, 18 presented high-grade fibrosis, while 42, low-grade fibrosis. The authors also observed that the duration of exposure to iron is important in the development of liver fibrosis in HH.

In 2006, Powell *et al*. (2006)[57] evaluated, by liver biopsy, 672 homozygous C282Y individuals. Through this study, they observed liver iron overload with grades 2 and 4, in 56% and 34.5% of males and females, respectively; liver fibrosis stage 2 and 4 in 18.4% and 5.4%; and cirrhosis in 5.6% and 1.9%. The study concluded that fibrosis and liver cirrhosis are significantly correlated with liver iron concentration.

CONCLUSION

Based on all of the above, it is clear that individuals with homozygous for C282Y genotypic diagnosis for HH associated with low expression of the *HFE* gene have higher levels of iron overload in the body than those with the C282Y/H63D mutation, and those not diagnosed with HH. It is known that liver tissue is the main site of iron deposition. In this context, we found that the higher levels of iron deposition in liver cells are significantly associated with the activation of the stellate cells, with consequent exacerbated collagen production and, subsequently, progression to hepatic fibrosis, thus proving its chronic association with the increased risk of developing more severe liver injuries, such as cirrhosis. Therefore, it is necessary to perform tests such as iron measurement, serum ferritin and Tf saturation to assess the body iron stores, aiming at an early diagnosis of iron overload in order to avoid deleterious effect to cellular and tissue levels.

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