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REVIEW ARTICLE

The role of hepcidin and haemojuvelin in the pathogenesis of iron disorders in patients with severe malnutrition

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Abstract

Introduction and objective. The clinical consequences of malnutrition are multi-directional and result in dysfunctions of the majority of internal organs and systems. The results of recent studies suggest that a significant role is played by malnutrition in pathophysiology of iron homeostasis disorders, but the underlying mechanism is unclear. The study describes the potential role of hepcidin and hemojuvelin in the pathogenesis of disorders of iron metabolism during malnutrition.

State of knowledge. The participation of hepcidin in regulating iron homeostasis encompasses inhibiting the absorption of food iron from enterocytes and inhibiting the release of stored iron from the reticuloendothelial system cells. One of the factors that increases the post-translational level is the expression of hepcidin is IL-6. In studies focused on malnutrition it was observed that in persons with protein and energy deficits the level of proinflammatory cytokine, i.e. interleukin-6, in the serum, increased. The involvement of haemojuvelin in the overall iron homeostasis is related with the regulation of expression of the hepcidin coding gene on the transcription level. The highest haemojuvelin expression was observed in humans in skeletal muscles. Observations and analyses conducted *in vivo* allowed the conclusion that soluble HJV and cell-related haemojuvelin regulate hepcidin expression in response to changes in iron concentration. The research also demonstrated that soluble HJV neutralizes the inductive effect of IL-6 action on hepcidin expression.

Summary. It can be claimed that in persons with protein and/or protein-energetic malnutrition the muscle mass deficit may lead to insufficient production of haemojuvelin and sideropenia.

Key words

hepcidin, haemojuvelin, iron disorders, malnutrition

INTRODUCTION

Malnutrition is defined as pathology resulting from either prolonged deficit of energy nutrients and/or microelements in one's diet or recurring infections or chronic diseases [1]. Etiology differentiates three types of malnutrition:

1) energetic (marasmus, cachexia), developing due to exposure to long-term energy deficit;
2) protein (kwashiorkor), resulting from insufficient protein consumption and/or catabolic stress related with infections and extensive traumas;,
3) mixed, i.e. protein energy malnutrition (PEM), most frequently observed in a variety of acute or chronic diseases [2].

While analyzing the causes of malnutrition, it should be borne in mind that especially in hospital patients it is of a secondary character and co-exists with many other diseases [3]. Reports also refer to cases in which the consequences of malnutrition are the initial symptom of another disease. Persons with high risk of developing severe malnutrition include patients suffering from cancer and gastrointestinal diseases, as well as psychological and neurological disorders [4, 5]. A high ratio of malnutrition occurrence is also reported in patients with hyperthyroidism and in patients suffering from post-traumatic stress [4, 5].

The clinical consequences of malnutrition are multi-directional and result in dysfunctions of the majority of internal organs and systems [6]. The clinical picture of malnutrition shows skeletal muscle atrophy, proliferation of extracellular space with a tendency for the occurrence of oedema, immunity decrease, deficient wound healing, susceptibility to decubitus ulcers, decrease of physical activity, fatigue, apathy and hypothermia [4, 6, 7]. Results of research in the literature suggest that a significant role is played by malnutrition in the pathophysiology of iron homeostasis disorders.

Iron disorders in severe malnutrition. The unquestionable relation between malnutrition and iron disorders (manifested in microcytic anaemia) seems to be quite well reflected in a number of researches conducted among children with severe malnutrition [8, 9, 10]. The research show a significant increase of ferritin level in the serum of children suffering from malnutrition with concurrent iron deficit, in comparison to children with sideropenia who are not suffering from malnutrition. [8]. Ferritin concentration increase was additionally accompanied by a significant reduction of transferin concentration and reduced TIBC (total iron-binding capacity). The research performed by Agarwali et al. [8] support the claim that malnutrition affects the development of iron disorders. Researchers showed that among such factors as infections, malnutrition, inflammation

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or proteinuria, malnutrition exerted the biggest influence on the reduction of transferin level and total iron-binding capacity [8]. Changes in protein plasma profiles probably result from the inflammatory process, co-occurring with malnutrition, in the course of which – at the expense of functionally important proteins, including transferin – acute phase proteins, i.e., ferritin, are synthesized. The results of the aforementioned research seem to be particularly adverse in view of the research conducted by Broxmeyer et al. [11]. The authors research conducted *in vitro* showed the inhibitive influence of ferritin on the proliferation of myeloid cells [11]. Six years later, a team led by Vreugdenhil proved that removing excessive ferritin from the body by using iron chelating substances results in the improvement of haematological test results [12].

The fact that malnutrition may play a vital role in the pathophysiology of anaemia was also suggested by Mitrache et al. who relied on the results of research conducted on 186 patients with an average age of 85 (between 56–100 years of age) [13]. The authors showed in the multi-factor regression analysis that anaemia was significantly related with a low albumin level in the serum [13]. Other observations made in this study addressed the fact that 42% of geriatric patients with anaemia also met the criteria for malnutrition.

The role of hepcidin and haemojuvelin in the pathogenesis of iron disorders in severe malnutrition. Hepcidin is a peptide with high cysteine content and of molecular weight of ca. 3 kDa that occurs in body fluids in three forms. In the serum, isoforms of 25 and 20 amino acids are identified, and a peptide consisting of 25 amino acids is dominant [14]. Shorter forms i.e., made of 20 or 22 amino acids are identified mostly in urine [14, 15]. The mature and biologically active form of hepcidin contains in its sequence 8 cysteine residues connected by disulfide bonds [16]; disulfide bonds stabilise the cell structure [17]. Results of a number of researches focused on hepcidin show that it is a key mediator involved in iron homeostasis. The participation of hepcidin in regulating the overall iron homeostasis encompasses inhibiting the absorption of food iron from the lumen of absorptive enterocytes, and inhibiting the release of stored iron from the reticuloendothelial system cells. Nemeth et al., using cell cultures, showed that hepcidin performs its physiological activity through reactions with ferroportin (Fpn), i.e. the transmembrane protein receptor [18]. Ferroportin is present on the surface of cells, releasing iron to the circulating pool, such as absorptive enterocytes, macrophage, hepatocytes, and placenta cells [18]. The biological function of ferroportin is the transportation of iron from the cell interior to the blood vessels. Hepcidin is a factor regulating ferroportin expression on the post-translational level [18, 19]. It shows the capacity of binding directly with ferroportin molecules; hepcidin-Fpn binding is then subject to internalisation. Inside the emergent endosome, ferroportin is subject to lysosomal degradation. The loss of the cell membrane surface ferroportin is a secondary constraint on the process of iron release from the cell interior [18, 19].

Regulation of expression of the hepcidin coding gene (*HAMP*), located on the long arm of chromosome 19 (19q13), occurs both on the transcription and post-translational level [17, 20]. One of the factors with reported influence on hepcidin expression on the post-translational level is interleukin 6 [21–25]. Interleukin-6 induces hepcidin expression, thus being a mediator in the development of hypoferremia [23, 24]. In studies focused on malnutrition it was observed that in persons with protein and energy deficits (without any infection features) the level of proinflammatory cytokine, *inter alia* interleukin-6, in the serum increases [9, 10, 26]. A negative correlation between IL-6 and the condition of the muscle mass was also demonstrated [26]. The research results presented above also support the claim about the essential role of malnutrition in the pathogenesis of iron homeostasis disorders.

The involvement of haemojuvelin in the overall iron homeostasis is related with the regulation of expression of hepcidin coding gene on the transcription level. Activation of the hepcidin coding gene on the transcription level occurs through two varying cellular signalling pathways: through activating Stat3 and through activating Smad proteins [20, 27, 28]. Pro-inflammatory cytokines, in particular interleukin-6, induce the transcription process of the HAMP gene through activating Stat3, and then binding Stat3 to the regulatory region in the *HAMP* promoter [20, 27, 28]. The other mechanism of hepcidin expression control depend on the BMP/Smad signalling pathway. The connection of bone morphogenetic protein (BMP) with type II (BMPR II) or type I receptors (BMPR I) (BMP-typeI/typeII) entails phosphorylation of RSmad intracellular protein, which is then bound with Smad4 (also described as Co-Smad) [20]. The emerging complex (RSmad-Smad4) is relocated to the nucleus where, interacting with other transcription factors, it induces target genes, including the hepcidin gene [20].

The latest research shows a very important role played by haemojuvelin (HJV) in regulating the transcription of the *HAMP* gene through the BMP/Smad pathway [29–31]. Haemojuvelin is a protein product of the *HJV* gene located on the long arm of chromosome 1 (1q21). The highest haemojuvelin expression was observed in humans in skeletal muscles, the myocardium, liver, oesophagus and pancreas. Initially, researchers proved that in *in vitro* conditions haemojuvelin acts as a co-receptor of bone morphogenetic protein which facilitates the activation of the BMP-type I/type II complex [29]. Subsequent meticulous observations and analyses conducted *in vivo* allowed the conclusion that soluble HJV inhibits hepcidin expression [30, 31]. Intraperitoneal injection of recombined soluble HJV into mice at the dose of 25mg/kg of bodyweight, three times per week for three consecutive weeks, resulted in the following: inhibition of hepcidin expression, increase in ferroportin expression and mobilization of stored iron from the reticuloendothelial system cells [30]. The final effect of the activity of exogenous soluble HJV was the iron increase in the serum [30].

The authors of the above-mentioned research also demonstrated that soluble HJV neutralizes the inductive effect of IL-6 action on hepcidin expression. Adding interleukin-6 into the hepatic cells (HepG2) culture increased hepcidin expression threefold [30]. The already observed and stimulating influence of IL-6 on the hepcidin synthesis was clearly neutralised when hepatocytes were incubated together with IL-6 and in combination with soluble HJV [30]. The research performed by Lin et al. demonstrated that soluble HJV and cell-related hemojuvelin jointly regulate hepcidin expression in response to changes in iron concentration in the extracellular space [31]. In view of the aforementioned biological activity of haemojuvelin, what seems particularly interesting are research results presenting increased release

of HJV by skeletal muscle cells in rats in response to iron deficits [32]. The authors of these reports suggest that skeletal muscles may perform a vital role in iron homeostasis [32]. It can be claimed that in persons with protein and/or protein-energetic malnutrition the muscle mass deficit may lead to insufficient production of haemojuvelin, thus yielding a secondary result of neutralising physiological response of the body to sideropenia.

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