Journal of Blood Disorders

Open Access

Austin Publishing Group

Special Article - Iron Deficiency Anemia

Hypothesis: Clinical Symptoms of Iron Deficiency – Evidence of Functional Inability of Nitric Oxide Petukhov VI

Petukhov VI*
Department of Psychology, Baltic International Academy, Latvia

***Corresponding author:** Petukhov VI, Baltic International Academy, LV-1003, Lomonosova St., 4, Riga, Latvia

Received: July 25, 2016; **Accepted:** August 11, 2016; **Published:** August 16, 2016

Abstract

This paper presents a hypothesis which explains the origin of sideropenic symptoms in Iron Deficiency States (IDS). According to the author, typical symptoms of IDS – namely, regenerative disorders, muscle weakness, cognitive impairments, or taste and smell perversions (pica chlorotica) – may be caused by functional inability of Nitric Oxide (NO).

Keywords: Nitric oxide; Iron deficiency; Criticality; Electronic Paramagnetic Resonance (EPR)

Abbreviations

ID: Iron Deficiency; IDS: Iron Deficiency States; IDA: Iron Deficiency Anemia; LID: Latent Iron Deficiency; NO: Nitric Oxide; Hb: Hemoglobin; DNIC: Dinitrosyl Iron Complexes; SC: Self-Organized Criticality; EPR: Electronic Paramagnetic Resonance; SERCA: Ca-ATPases of the (endo-) Sarcoplasmic Reticulum; RyR: Ryanodine Receptor; EP: Electric Potential; AP: Action Potential

Short Communication

The clinical picture of Iron Deficiency (ID) is characterized not only by varied and exotic symptoms (*e.g., pica chlorotica*) but also by lack of indisputable interpretation of the origin of these symptoms. For example, for an explanation of muscle weakness, one of the most typical signs of ID, one usually refers to the large family of Fe-containing enzymes, whose activity, supposedly, directly depends on the level of iron in the body. However, despite the 'survivability' of this hypothesis, which has been around for decades, it was never confirmed unambiguously.

Indeed, following this concept, it is difficult to understand why, even with a moderate Fe-deficiency, when the hemoglobin level is still within the normal range (while at least 70-75% of the total iron is spent on its maintenance); Fe-containing enzymes (whose need for this metal is less than 1%) are already experiencing the lack of Fe? Why not all the Fe-dependent enzymes are equally susceptible to iron deficiency (*e.g.*, catalase, the content of which may remain unchanged even in severe iron deficiency)? Many contradictions can be found in the explanation of the causes of cognitive and regenerative disorders inherent in ID, which are often associated with '*tissue hypoxia*', which is determined 'by eye' (without further specification).

Therefore, the search for new approaches to explain the genesis of ID clinical signs is very opportune. One approach (as a hypothesis) is presented in this paper.

ID is conventionally divided into express Iron Deficiency Anemia (IDA) and Latent Iron Deficiency (LID). The latter is diagnosed under normal levels of hemoglobin (Hb) and red blood cells but reduced levels of serum ferritin (<15-20 ng/ml) and increased blood content of soluble transferrin receptor (sTfR > 8.5mg/l). Clinical manifestation of ID can be represented in the form of the following major groups of sideropenic symptoms:

1. Reduced muscle contractile capacity (regardless of muscle type). The sign is characteristic of IDA and not found in other anemias.

2. Disturbed regeneration of cover tissue (skin and its appendages, the mucosa of the gastrointestinal tract, etc.), when the decline of cells (apoptosis) quantitatively exceeds cell reproduction.

3. Cognitive disorders (pathognomonic for ID).

4. Taste and smell perversion (*pica chlorotica*). Close association of these symptoms with ID is used in the differential diagnostics of anemias.

Symptoms of hemodynamic disturbances: tachycardia (even after slightest physical exertion), dizziness, fainting, headache, etc., which are inherent not only in IDA, but in all anemic syndromes without exceptions, are not included in the above list since they have a common and understandable reason – hemodilution.

These four groups of sideropenic pathognomonic symptoms seem dissimilar, but only at first glance. All of them (at least supposedly) may have common origin or pathogenesis, which is based on the lack of Nitric Oxide (NO) ability (bioavailability), causally related to ID. Arguments in favor of this assumption (for each group) are presented in this article. But first I would like to explain why the functional activity of nitric oxide may depend on the level of iron in the body.

It is known that NO is a short-lived molecule (lifetime is measured in milliseconds). This fact is not in any way consistent with the known ability of NO to perform a huge amount of work as a universal manager. Therefore, it is reasonable to assume the existence of a special mechanism, which imparts the long-term and distant character to the mediating function of nitric oxide.

According A.F. Vanin *et al.* [1], the role of such 'mechanism' can

Citation: Petukhov VI. Hypothesis: Clinical Symptoms of Iron Deficiency – Evidence of Functional Inability of Nitric Oxide Petukhov VI. J Blood Disord. 2016; 3(1): 1039.

be attributed to the three-component chemical system (NO, non-heme Fe^{2+} and low molecular weight thiols) which is permanently functioning in autocatalytic mode (the Belousov-Zhabotinsky reaction type). In this system interconversion occurs between Dinitrosyl Iron Complexes (DNIC) with thiol-containing ligands ($\{(RS^-)_2 Fe^+(NO^+)_2\}^+$), which are formed in the system, and S-nitrosothiols (RS-NO). Given sufficient supply of necessary components (NO, thiols, Fe^{2+}), the system is a long-term and distant (in case of circulating cells) source of nitric oxide, more precisely, its ionized and more reactogenic forms – nitrosonium (NO^+) and nitroxyl (NO^-). The NO^+/NO^- ions are capable of activating the membrane ATPases by nitrosation of thiol groups of proteins, thereby causing changes in the metal content in the cell, for example, Ca^{2+} [2,3].

However, the self-oscillating mode of operation of these systems, if they occur in normal conditions (i.e. at a sufficient density distribution in the cell), must inevitably lead to synchronization of their oscillations or using the term of the Self-organized Criticality (SC) Theory to the *critical state* of the oscillators system [4].

The main criterion of the *critical state* for the oscillators system is a branching parameter (σ), by which you can judge about the nature of excitation (energy) spread in the oscillator network. It is numerically equal to the average number of 'neighbors', to which energy is conveyed by each oscillator. In this case (i.e. critical state), the transfer of energy would mean synchronization of self-oscillations of the neighboring oscillators and the system as a whole; as a critical (synchronized) state σ = 1 (i.e. excitation is transmitted in average to one adjacent oscillator).

Apart from being in critical state, the system may also be in subcritical (σ<1) or supercritical (σ>1) state, demonstrating desynchronization of oscillators' operation. It can be assumed that the lack of iron, leading to a decrease in the number of ($\{(RS^-)_2 Fe^+(NO^+)_2\}^+$)/(RS-NO)-oscillators, will cause the system to switch to subcritical (σ <1) state (desynchronization) with the consequent reduction of NO viability.

The results of our follow-up of ID patients showing significantly reduced NO level in epidermal cells (EPR analysis data) confirm the reality of these events [5]. Besides, a marked positive correlation (Pearson) was found between the level of serum ferritin (the main indicator of iron sufficiency) and NO-signal magnitude on the EPR spectrogram (r=0.49; p <0.05) [5].

The clinical analogue of *supercritical state* (σ>1) can be overdose of nitrates and the related sharp decline in vascular tone, as well as collapse with all kinds of shock caused by the overproduction of endogenous nitric oxide. Interestingly, the injection of methylene blue solution (for binding of excessive NO to relieve the nitrosative stress) can restore the normal tone and peristalsis of blood vessels, possibly due to the recovery of the critical or near-critical state (synchronization).

It is highly probable that the system functioning near the critical level (σ = 1) provides normal muscle contraction and relaxation not only of cardiomyocytes but also of myocytes in blood vessels. This assumption needs to be clarified.

It is known that muscle contraction is required not only by ATP but also by Ca^{2+} ions, whose transmembrane traffic is ensured by a number of membrane pumps: Ca-ATPases of the plasma membrane and the (endo-) Sarcoplasmic Reticulum (SERCA), ryanodine receptor (RyR), Na/Ca exchanger, and others. In this case, normal (synchronous) operation of these pumps may be largely determined by the adequate production of NO^+/NO^- ions [3].

The modern idea of the nature of the cardiac automatism allows for the existence of two synchronous generators of Electric Potential (EP) in pacemaker cells and cardiomyocytes. This is the so-called 'membrane clock' in the outer membrane of the cell and 'calcium clock' in the membrane of the Sarcoplasmic Reticulum (SR). EP production is ensured by active transmembrane Ca^{2+} traffic with participation of the abovementioned pumps operating, according to the researchers, in the automatic mode [6,7]. The role of pacemaker in this process is attributed to the 'calcium clock', whose relations with the 'membrane clock' are based on the subordination principle. We do not exclude, however, that the role of pacemaker (*the order parameter*) may belong to NO^+/NO^- - the generating system of ($\{(RS-)_2 Fe^+ (NO^+)_2\}^+$) / (RS-NO)-oscillators. The chain of possible events is viewed as set out below.

Formation NO^+/NO^- (in the self-oscillation mode) causes alternant synchronous S-nitrosation of the thiols in the molecules of ATP-as and RyR2 followed by induction of transmembrane traffic of electrogenic metals (notably Ca^{2+}). This leads to the appearance of the Action Potential (AP) and the alternation of muscle contraction (activation of RyR2 and output of Ca^{2+} from the SR to the cytosol) and muscle relaxation (SERCA activation and reverse pumping of Ca^{2+} from the cytosol to the SR).

In such case, the working rhythm of the 'calcium clock' and 'membrane clock' will depend on the simultaneous ('salvo') occurrence of a sufficient amount of NO (NO^+/NO^-) in the cell for the S-nitrosation of proteins in the membrane pump structure and the proper transmembrane traffic of Ca^{2+}. For the number of working (NO^+/NO^-)-molecules to be sufficient, synchronization of self-oscillations in the system of ($\{(RS^-)_2 Fe^+ (NO^+)_2\}^+$)/(RS-NO)-oscillators must be high (at least *critical*). Regarding the specific mechanisms that are responsible for the coherence of muscle contraction/relaxation, it is not very clear how it works. One can hardly doubt, however, the necessity of critical state (synchronization) as the main factor of the existence of these processes (not only in cardiac myocytes and myocytes of blood vessels but also in the smooth muscle cells of hollow organs). Therefore, we may consider the contraction/relaxation of cardiomyocytes and smooth muscle cells as a SC-phenomenon.

The idea of the critical state of the system (NO^+/NO^-) - generating oscillators helps better understand the therapeutic effect of nitrates (such as nitroglycerin). It is no secret that the antianginal effects of the latter (as NO donator) is almost always associated with vasodilatation, forgetting (or not mentioning) the known inotropic effect of nitroglycerin, which is hardly limited to cardiomyocytes alone and does not affect vascular myocytes simultaneously.

One cannot exclude that additional (with NO-containing drugs) acceptance of molecules of nitric oxide, by increasing the concentration of (NO^+/NO^-)-generating oscillators, leads not only to

their synchronization (critical state) but also to the normalization of Ca^{2+} traffic in vascular myocytes. This, in turn, may contribute to the restoration of full relaxation and, just as importantly, to contraction of muscle cells, or, in other words, normalization of vascular peristalsis as a prerequisite of effective myocardial perfusion in the ischemic area.

The emergence of subcritical ($\sigma<1$) state for the (NO^+/NO^-)-generators in atherosclerosis is to be expected (at least locally, in the most affected areas of vascular network). Therefore the supplement of an external NO (nitroglycerine) for these areas becomes imperative, because it is helping to change the state of the system, making it critical. The clinical experience may testify the reality of such events.

It is known that with the beginning of IDA in patients with angina pectoris, the number of attacks of chest pain, as well as a daily dose of nitroglycerin, increases markedly. But a few days after the beginning of reception of Fe-containing drugs, these attacks become less frequent, though Hb level remains lower than norm. Non-heme iron, as an essential component of the self-oscillation complex - $(\{(RS^-)_2 Fe^+ (NO^+)_2\}^+)$/(RS-NO), being in short supply, can apparently, cause desynchronization of (NO^+/NO^-)-generators because of decline in the number of the latter (subcritical state). Therefore, antianginal (synchronizing) effect of iron supplementation precedes restoration of normal Hb level.

As indirect confirmation of the wave-like (self-oscillating) nature of the events occurring in cardiomyocyte matrix, one can mention the clinical observations of patients with coronary heart disease, whose anginal syndrome was successfully arrested without additional introduction of nitric oxide (in the form of nitrates). That was possible due to the fact that the clinic had been using (for over 10 years) electromagnetic waves in the terahertz range (100 GHz to 10 THz) frequencies of molecular spectrum of nitric oxide [8]. According to the authors, the pain control was associated with increased NO power by lengthening the term of active intermediary activity. We cannot exclude that the terahertz waves can promote synchronization of self-oscillations of (NO^+/NO^-) -generators, i.e., cause changeover of the system's subcritical condition to critical.

Apparently, the critical processes (synchronization) play not a lesser role in contraction/ relaxation of skeletal muscles than in the smooth muscle cells and cardiac myocytes. The absence of a specified rate of contractions/relaxations does not exclude the criticality (synchronization) of self-oscillations in the system of (NO^+/NO^-)-generators as a prerequisite for the normal (coherent) Ca^{2+}-traffic in skeletal myocytes. In other words, iron deficiency in the body may cause the subcritical state of the system, which is associated with inevitable skeletal muscle dysfuncion.

Regenerative disturbances of cover tissues associated with Iron Deficiency States (IDS) are most probably attributable to the following two factors. First, pro-apoptotic effect of peroxynitrite ($ONOO^-$), whose elevated content (by nitrotyrosine level) was found in iron deficiency [9]. Second, reduction in antiapoptotic activity of nitrosonium ions (NO^+) due to their insufficient production in Fe-deficiency conditions (subcritical state of the NO^+-generating system). Anti-apoptotic effect of NO^+ is associated with its ability to S-nitrosylate cysteine at the catalytic center of caspases that are involved in apoptosis. This causes inactivation of the said enzymes. It was shown that NO^+ is capable of reversibly inhibiting the seven kinds of recombinant caspases [10].

Activation of apoptosis in ID may indicate an increased release of cytochrome-C from mitochondria of cardiomyocytes, as well as increased content of caveolin-1 (a marker of apoptosis) in these cells in Fe-deficient rats [9]. Those observations suggest a possible link between the subcritical state of the oscillators system and the impaired regeneration of cover tissues.

The occurrence of cognitive disorders against the background of insufficient NO-production is well known. According to the authors [11] (cit.) '*NO provides an isolation of neurotransmitters in the central nervous system, involved in the synaptic transmission and in the formation of long operating connections between neurons - the synaptic potentiating that underlies learning and memory*'. In the experiments on rats, it was shown that nitroxide can prevent cognitive disorders in neurodegenerative lesions of the brain [12]. Thus, we cannot exclude that decline in the NO-efficiency (subcritical state) against the background of Fe-deficiency plays a significant role in the genesis of cognitive disorders.

The relationship between nitric oxide and olfactory brain function is under investigated. This issue needs to be studied in greater depth. However, the available data on the substantial content of NO in the olfactory bulb [13] and nitric oxide participation in the formation of the olfactory memory allow the following assumption. Iron deficiency, which is associated with disorders of smell and taste (*pica chlorotica*), facilitates transition of the oscillators system to subcritical state and causes functional NO-failure, which, most probably, explains the origin of these symptoms.

Thus, the idea of synchronization (criticality) of processes underlying intermediary nitroxide function suggests (as a hypothesis) the new interpretation of sideropenic symptoms in patients with IDA.

References

1. Vanin AF, Papina AA, Serezhenkov VA, Koppenol WH. The mechanism of S-nitrosothiol decomposition catalyzed by iron. Nitric Oxide Biol Chem. 2004; 10: 60-73.
2. Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, et al. Nitroxyl Improves Cellular Heart Function by Directly Enhancing Cardiac Sarcoplasmic Reticulum Ca^{2+} Cycling. Circ Res. 2007; 100: 96-104.
3. Lancel S, Zhang J, Evangelista A, Trucillo MP, Tong X, Siwik DA, et al. Nitroxyl activates SERCA in cardiac myocytes via glutathiolation of cysteine 674. Circ Res. 2009; 104: 720-723.
4. Bak P. How Nature Works. The science of self-organized criticality. Copernicus, New York. 1996.
5. Petukhov VI, Baumane LK, Reste ED, Zvagule T, Romanova MA, Shushkevich NI, et al. Diagnosis of nitrosative stress by quantitative EPR-spectroscopy of epidermal cells. Bull Exp Biol and Med. 2012; 154: 698-700.
6. Lakatta EG, Maltsev VA, Bogdanov KY, Stern MD, Vinogradova TM. Cyclic variation of intracellular calcium: a critical factor for cardiac pacemaker cell dominance. Circ Res. 2003; 92: e45-e50.
7. Maltsev VA, Lakatta EG. Normal heart rhythm is initiated and regulated by an intracellular calcium clock within pacemaker cells. Heart Lung Circ. 2007; 16: 335-348.
8. Parshina SS, Afanasjeva TN, Tupikin VD, Kirichuk VF, Ostrovsky NV, Vodolagin AV. Nitric oxide and terahertz radiation: perspectives of clinical use. In book: New Information Technology in Medicine, Pharmacology, Biology and Ecology, IT+M&E Press, Yalta-Gurzuf. 2013; 112-115.

9. Dong F, Zhang X, Culver B, Chew HG, Kelley RO, Ren J. Dietary iron deficiency induces ventricular dilation, mitochondrial ultra structural aberrations and cytochrome *c* release: involvement of nitric oxide synthase and protein tyrosine nitration. Clinical Science. 2005; 109: 277-286.

10. Li J, Billiar TR, Talanian RV, Kim YM. Nitric oxide reversibly inhibits seven members of the caspases family via S-nitrosylation. Biochem Biophys Res Commun. 1997; 240: 419-424.

11. Shupik AI, Vanin AF, Alesenko AB. The interaction of nitric oxide signaling system with sphingomyelin cycle and during the peroxide oxidation signal toxicity of tumor necrosis factor α in ischemia - reperfusion. Biochemistry. 2011; 76: 1489-1504.

12. Menuhin EB, Pšennikova MG, Gorači AV, Khomenko IP, Pokidyšev DA, Malyshev IU. Role of nitric oxide in the warning kognitivnyh Reputation nejrodegenerativnom Damage in brain krys. Bull exp biol med. 2008; 10: 371-375.

13. Varner PD, Beckman JS. Nitric Oxide in the Nervous System. Vincent SR, Editor. London. 1995; 191-206.

J Blood Disord - Volume 3 Issue 1 - 2016
ISSN 2379-8009 | www.austinpublishinggroup.com

Citation: Petukhov VI. Hypothesis: Clinical Symptoms of Iron Deficiency – Evidence of Functional Inability of Nitric Oxide Petukhov VI. J Blood Disord. 2016; 3(1): 1039.