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Mitochondriopathy caused by NTBI overload: the role of piracetam in the most problematic stage of H63D syndrome

Abstract

This paper presents an in-depth discussion surrounding the potential implications of chronic non-transferrin bound iron (NTBI) overload, mitochondrial dysfunction, and the utilization of piracetam as a potential therapeutic approach in H63D syndrome. Starting from a patient scenario with iron overload secondary to a homozygous HFE gene H63D mutation and transferrin saturation constantly >50%, we explore the pathological mechanisms involving iron metabolism, oxidative stress, and cellular damage, with a particular focus on mitochondrial injury. The paper delves into the potentially complex interactions between iron, reactive oxygen species, and the mitochondrial respiratory chain, with an emphasis on how continued iron overload can lead to disease progression despite dietary interventions. It also speculates on the potential role of piracetam as a therapeutic option, discussing its possible mechanisms of action and considering the risk-benefit balance. Lastly, it underscores the complexity of iron overload conditions and the need for ongoing research into effective treatment strategies.

The NTBI cascade into H63D Syndrome

The small circle of clinicians who treat "rare diseases" have been aware for many years of the problematic fact that chronic transferrin saturation of >50% (fasting and/or in the postprandial state) leads to non-transferrin-bound iron (NTBI), a highly toxic entity. The disease caused by this is H63D

syndrome type-1, however, this is only the beginning of a cascade of severe multi-organ conditions that will almost inevitably lead to a severe mitochondriopathy if not treated in time with a strict low iron diet that must reduce transferrin saturation to below 45% at the very latest before the onset of puberty.

Transferrin saturation (TFsat) is a blood test that measures the percentage of transferrin - a protein that carries iron in the blood - that is loaded with iron. A normal transferrin saturation value ranges from about 20% to 45% (Brissot et al, 2001)

If the transferrin saturation is consistently above 45-50%, it could be indicative of iron overload conditions such as:[7-56]

1. **Hemochromatosis**: This is a hereditary condition where the body absorbs too much iron from the diet, leading to iron buildup in the body. The excess iron can deposit in various organs, including the liver, heart, and pancreas, potentially leading to conditions such as cirrhosis, heart disease, and diabetes.
2. **H63D Syndrome:** Also a hereditary iron metabolism condition which leads to an insidious overload with NTBI iron (explanation below)
3. **Iron-loading anemias**: These are conditions like thalassemia and sideroblastic anemia where ineffective erythropoiesis leads to increased iron absorption and subsequent iron overload.
4. **Excess iron supplementation or repeated blood transfusions**: People who receive multiple blood transfusions or consume excessive iron supplements can also experience iron overload.

Persistently high transferrin saturation is a concern because iron is a pro-oxidative element, meaning it can generate free radicals that damage cellular structures, including DNA, proteins, and lipids. Over time, this oxidative stress can contribute to a variety of health problems, including liver disease, heart problems, diabetes, arthritis, and even certain types of cancer. That is why it's important for people with consistently high transferrin saturation to work closely with their healthcare provider. They may require further diagnostic tests and potentially treatments to reduce iron levels and monitor for complications. Treatment for iron overload typically involves phlebotomy (removing blood from the body), iron chelation therapy (medication that binds to and removes excess iron), or adjustments to the diet or iron supplementation regimen (Ivanova et al., 2022).

Persistently high transferrin saturation, above 50%, indeed indicates a state of iron overload. Iron, as every doctor and PR guy working for the food industry knows, is essential for various biological functions, including oxygen transport, DNA synthesis, and electron transport. However, its ability to cycle between different oxidative states can lead to the formation of reactive oxygen species (ROS), which can damage cellular components and contribute to various pathologies.[3-7,12,27-38]

Iron overload affects numerous metabolic pathways and can result in a variety of cellular dysfunctions. In the liver, it can lead to hepatocyte damage, NAFLD, NASH, cryptic inflammation, fibrosis of all degrees, and ultimately cirrhosis and hepatocellular carcinoma due to ROS (Valenti et al., 2010) Moreover, excess iron in hepatocytes can inhibit hepatic hepcidin synthesis, which would further exacerbate the systemic iron overload.[37,38,42,53]

In the heart, excess iron can induce cardiomyocyte apoptosis, fibrosis, and alter cardiac conduction (red flags: AV and/or all types of bundle branch blocks), leading to arrhythmias, dilated cardiomyopathy, cryptic changes of the heart function

(visible in ECG or TTE), as well as chronic heart failure. Iron overload in the endocrine system can cause β-cell dysfunction in the pancreas leading to diabetes mellitus, and it can disrupt the hypothalamic-pituitary axis, causing hypogonadotropic hypogonadism (Gunel et al., 2009). From a diagnostic point of view, persistently high transferrin saturation, along with high serum ferritin, could would be suggestive of hereditary hemochromatosis, especially in the presence of symptoms or a positive family history. However, the final diagnosis would need to be confirmed through genetic testing for C282Y and H63D mutations in the HFE gene. It is also important to consider differential diagnoses such as iron-containing anemias and many rare diseases. In particular, it is of utmost importance to know as early as possible whether a person has a homozygous mutation of the HFE gene H63D, as this gene can cause even greater damage than hereditary hemochromatosis ever could in a subset of patients due to H63D syndrome. It is still not entirely clear why this fate affects only around 10% of mutation carriers, with a second hit (often a severe infection) being the most likely explanation.[2,4,5,6,9,17,38,42]

The fall from the H63D cliff

What makes a homozygous mutation of the HFE gene H63D so extraordinarily explosive? Short answer: an abnormally functioning transferrin system. Ferritin is low, but TFsat is high. This leads to a catastrophic health condition affecting multiple organs: non-transferrin-bound iron (NTBI) refers to the pool of iron in the blood that is not bound to the primary iron-transporting protein, transferrin. Under normal physiological conditions, virtually all serum iron is bound to transferrin, which safely transports iron to cells throughout the body. But when transferrin becomes saturated due to an excess of iron, additional iron appears in the serum as NTBI.[72] This very type of iron is extremely harmful because it can be taken up more rapidly by cells than transferrin-bound iron, leading to a build-up of iron within tissues and organs. This is because NTBI uptake is not as tightly regulated as transferrin-bound iron, which is mediated by the transferrin receptor. NTBI is particularly taken up by the liver and heart, which can lead to conditions like liver cirrhosis and heart disease.[25,48]

In the context of a homozygous HFE gene H63D mutation and a compromised transferrin system, this could potentially lead to a situation where transferrin saturation is high but ferritin is low. Ferritin is a cellular protein that stores iron and releases it in a controlled fashion, and its levels in the blood can act as an indicator of the total amount of iron stored in the body.

If the transferrin system is compromised, ferritin might be low due to inadequate iron storage despite the presence of high amounts of circulating iron (hence the high transferrin saturation). Large quantities of iron might exist as toxic NTBI, which can profoundly contribute to the pathology of iron overload.

It is also worth noting that a homozygous H63D mutation in the HFE gene is often mistakenly considered to be less severe than a C282Y mutation simply because it causes far fewer of classic hereditary hemochromatosis in Caucasian people. In fact, in the form of H63D syndrome, it induces a disease many times more catastrophic for the patients than hereditary hemochromatosis. The phenotype can be highly variable, with some individuals displaying signs of various types of iron overload and others remaining almost asymptomatic.

Of course, this is a complex situation that would require a comprehensive clinical assessment, taking into account all

available clinical, laboratory, and genetic information, to make a proper diagnosis and develop an appropriate management plan. What is often found is an impaired transferrin system with the patient having a low ferritin but a TFsat of approximately 50 to 95%. In such a scenario, which is reality in our hospitals, where transferrin saturation is high (above ~50%) but ferritin levels are low, it indicates that there's a substantial amount of iron circulating in the blood, but the iron stores in the body (which ferritin levels often reflect) are low. This situation could potentially lead to excess non-transferrin bound iron (NTBI) in the bloodstream, as the transferrin system seems to be impaired and unable to efficiently bind and transport the circulating iron to cells for storage. As mentioned earlier, NTBI is potentially harmful because it can be taken up by cells more rapidly and in an unregulated manner compared to transferrin-bound iron. This rapid, unregulated uptake of iron can cause a buildup of iron within tissues and organs leading to iron-induced cellular damage due to oxidative stress.[2-14,57]

The clinical implications of this scenario would depend on several factors, including the extent of the transferrin system impairment, the degree of NTBI presence and cellular uptake, and the specific organs/tissues affected. Over time, however, this iron overload situation could potentially lead to organ damage and associated conditions, including liver disease (NAFLD, NASH, cirrhosis, and in a worst case scenario even hepatocellular carcinoma), heart disease (cardiomyopathy, conduction disorders, chronic progressive heart failure), endocrine disorders (diabetes type 3-C according to the European nomenclatures, hypogonadism), and others.[27,58,72]

Given the complexity of the situation, the management of this patient would likely require a personalized approach, which may include careful monitoring of the patient's iron status, potential use of iron chelators to reduce NTBI levels, and targeted interventions to address any organ damage that may occur due to iron overload. Research is ongoing into the best ways to manage conditions involving NTBI, as this is a complex and relatively recently understood aspect of iron metabolism.[2,36,55]

The consequences of a diagnosis made too late

Even in 2023 most physicians, even the highly reputable ones, do not have this potentially catastrophic consequences is mind. Still damages are being found in the Substania nigra of the brain, the heart, the liver, the spleen, the gonades, etc. In some patients you will see a relative eosinophilia relative basophilia. MCH and MCV might be a tiny bit low despite the patient has been put on a low-iron diet and TFsat is lower than before. But disease progression is still ongoing (brain, heart, gonades, eyes, neurodegenerative processes, etc.) - Why does the progression not stop?

The mitochondrial cliff

What then follows is a nightmare scenario for any clinician and, of course, first and foremost for the affected patient; a process indicative of systemic iron overload with multi-organ involvement and impaired mitochondrial function. Neurodegenerative changes, particularly in the substantia nigra, could indicate iron deposition as seen in well-known neurodegenerative disorders like Parkinson's disease. Heart and liver damages are common and potentially dangerous consequences of systemic iron (NTBI) overload. Relative eosinophilia and basophilia could be suggestive of an ongoing inflammatory or immune response. A slightly low MCV and MCH at times could be indicative of a mild microcytic, hypochromic anemia, perhaps due to some

chronic disease process or subtle iron utilization issues at the level of the bone marrow. The use of a low-iron diet in early stages of H63D syndrome and the subsequent reduction in transferrin saturation suggest that dietary iron can contribute to the overload and be controlled to some extent . However, a continued progression of the disease despite a strict diet hints at other factors at play.

Here are some possible reasons why the progression of the disease may never stop (Krone, 2022):

1. **Continued iron release from body stores**: Despite dietary modification, if there is a significant amount of iron already stored in the body (particularly in the form of hemosiderin in the reticuloendothelial system), it may continue to be mobilized and contribute to the iron overload state. Even if ferritin levels are low, there could still be iron deposits in tissues that aren't reflected in serum ferritin levels.
2. **Continued uptake of NTBI**: If the transferrin system is impaired, as suggested, then iron in circulation could still exist as NTBI. This can be taken up by cells in an unregulated manner, potentially leading to ongoing iron-induced cellular damage.
3. **Ineffective chelation of NTBI**: Even if dietary iron is limited, iron chelation therapy may be needed to remove NTBI. However, it's worth noting that NTBI chelation is a complex issue, as not all chelators are equally effective at removing different forms of NTBI. Moreover, iron chelation therapy could have its own highly severe risks and side effects.
4. **Ongoing damage from previous iron overload**: The effects of iron overload can be long-lasting. Even after iron levels are brought under control, there may be ongoing damage due to earlier iron-induced injury to cells and tissues. Oxidative stress, inflammation, and fibrosis caused by prior iron overload can continue to progress.
5. **Genetic factors**: The presence of the H63D HFE mutation and any other genetic factors could influence the rate of disease progression and the response to treatment.

This is indeed a complex and challenging situation that would likely require a multifaceted approach to management, including dietary modification, potential iron chelation therapy, and interventions to address the specific organ damages. Furthermore, given the potential role of inflammation and oxidative stress in the disease progression, anti-inflammatory and antioxidant therapies might also be beneficial. However, given the complexity and variability of iron metabolism and the potential for individual differences in disease progression and response to treatment, it is difficult to predict outcomes or treatment responses with certainty.

Mitochondriopathy: game over?

The most disastrous consequence of NTBI accumulation/ H63D mutation syndrome is the development of an acquired (sometimes called “secondary”) mitochondrial disorder which contributes to ongoing disease progression, even following the implementation of a well-arranged low-iron diet. Once started it is like a wildfire that will get out of control faster than the wind. Mitochondria are particularly susceptible to damage from reactive oxygen species (ROS) due to their role in oxidative phosphorylation and the consequent production of ROS. Long-term exposure to high levels of ROS - as could occur in a state of chronic iron overload - can lead to oxidative damage to mitochondrial

proteins, lipids, and DNA, impairing mitochondrial function.

Mitochondrial dysfunction can have wide-ranging effects on cellular function and metabolism due to the critical roles that mitochondria play in energy production, apoptosis, calcium homeostasis, and other crucial cellular processes. Consequently, mitochondrial dysfunction may contribute to the development and progression of a variety of H63D syndrome symptoms, including neurodegenerative disease, cardiovascular disease, and liver disease, which occur in H63D patients once they fall off the mitochondriopathy cliff. Furthermore, impaired mitochondrial function can exacerbate oxidative stress, creating a vicious cycle of mitochondrial damage and ROS production. This can lead to further cellular damage and contribute to ongoing disease progression, even if the initial source of excess iron (i.e., dietary iron) has been controlled. Additionally, the presence of mitochondrial disease could impact the efficacy of iron chelation therapy. Some iron chelators work in part by reducing iron-induced mitochondrial dysfunction. However, if the mitochondria are already significantly damaged, these agents might be less effective. Therefore, in these highly complex cases, management might need to address not only the iron overload but also the mitochondrial dysfunction, potentially with the use of mitochondrial-targeted antioxidants or other therapeutic strategies aimed at restoring mitochondrial function. However, like many aspects of these cases, the treatment of mitochondrial disease is a highly challenging area with many ongoing research and developments.[1-56]

Mitochondrial complexes

The mitochondrial respiratory chain, also known as the electron transport chain, is composed of four complexes (I, II, III, and IV) and ATP synthase (Complex V). Each plays a specific role in cellular respiration and ATP production. Iron overload, particularly in the form of non-transferrin bound iron (NTBI), can cause damage to these mitochondrial complexes through the generation of reactive oxygen species (ROS), primarily through the Fenton and Haber-Weiss reactions. The increased production of ROS can lead to oxidative damage to mitochondrial proteins, lipids, and DNA, potentially disrupting the function of the respiratory chain and decreasing ATP production. Among these complexes, Complex I and Complex III are considered major sites for ROS production within the mitochondria, particularly under conditions of iron overload. These complexes contribute to the generation of superoxide anion (O2.-), which can be further converted to other ROS. Therefore, in the context of long-term NTBI overload, Complexes I and III would be particularly susceptible to iron-induced oxidative damage. However, it is important to note that the exact extent and pattern of damage can vary depending on several factors, including the specifics of the iron overload condition, the cell types involved, and individual patient factors. Complex II and Complex IV can also be affected, although they are considered less significant sources of ROS. The resulting mitochondrial dysfunction could contribute to a variety of pathological conditions, depending on which cells and tissues are most affected. For instance, neurons and cardiomyocytes, which are highly dependent on mitochondrial ATP production, are particularly vulnerable to mitochondrial dysfunction, potentially contributing to neurodegenerative and cardiovascular conditions.[7,40-69]

Could Piracetam make a difference?

Piracetam is a medical drug that was developed many decades ago as a nootropic substance and has never become popular in Europe (where it remains

available) and North America due to inept marketing and pressure from competing products. However, it is still widely used in the rest of the world to improve cognitive function in patients with brain damage and some rare forms of dementia. Although the exact mechanisms of action are not fully understood, it is thought to have a number of interesting effects that could potentially impact the aforementioned targets related to iron overload and mitochondrial dysfunction - a classic win-win situation. Here is a brief overview of some of the proposed actions of piracetam:[71,74,75]

1. **Improving mitochondrial function and ATP production**: Some studies have suggested that piracetam can enhance mitochondrial function and ATP production. This could potentially help to counteract the mitochondrial dysfunction caused by iron overload.
2. **Protecting against oxidative stress**: Piracetam has been found to have antioxidant effects, which could help protect against the oxidative stress caused by iron overload. This might be particularly beneficial in the context of iron-induced damage to mitochondrial Complexes I and III.
3. **Enhancing neuronal function and survival**: Piracetam is thought to have neuroprotective effects, possibly through mechanisms such as enhancing membrane fluidity, improving neurotransmission, and promoting neuroplasticity. This could potentially help to counteract the neurodegenerative effects of iron overload.
4. **Modulating calcium homeostasis**: Piracetam has been shown to influence cellular calcium levels, which can play a role in numerous cellular processes and can be affected by iron overload.

While these effects suggest that piracetam may have some beneficial effects in the context of iron overload and mitochondrial dysfunction, it should be noted that the use of piracetam in this context has not been well studied. On the other hand, there is no other drug on the market that is at least theoretically as well suited to treat a mitochondriopathy caused by H63D syndrome as is piracetam.

The exact effects would likely depend on a variety of factors, including the specifics of the iron overload condition, the extent of the mitochondrial dysfunction, and individual patient factors. In terms of piracetam potentially causing harm in a situation of non-transferrin bound iron (NTBI) induced mitochondriopathy, there are no well-documented cases or studies directly linking piracetam to harm in this specific context. That said, the absence of evidence is not evidence of absence, so it is possible that such harm could occur under certain circumstances. Theoretically, there are a few ways in which piracetam might potentially cause harm:

1. **Overstimulation**: Piracetam is a psychostimulant and cognitive enhancer. In cases of severe mitochondrial disease, particularly if there's involvement of the heart or nervous system, overstimulation could potentially exacerbate symptoms or cause harm. However, piracetam is generally considered to have a low risk of overstimulation compared to other psychostimulants.
2. **Interaction with other medications**: Piracetam could potentially interact with other medications that a patient with NTBI induced mitochondriopathy might be taking. For example, there could be interactions with iron chelators or other medications used to manage the symptoms of mitochondrial disease.
3. **Unmasking latent conditions**: If a patient has an underlying genetic predisposition to seizures or other neurological conditions, piracetam might theoretically unmask these

conditions, although this is more speculative and would likely be rare.

Discussion

In the absence of specific evidence or guidelines, the decision to use piracetam in this context would likely need to be made on a case-by-case basis, taking into account the potential risks and benefits, the patient's overall clinical situation, and the presence of any other treatment options. As always, close monitoring would be necessary to detect any adverse effects early. It is also worth noting that our understanding of both NTBI induced mitochondriopathy and the effects of piracetam is still evolving, and new information could emerge that would influence this assessment. It's always important to stay updated with the latest research and guidelines in this area. However, when discussing overstimulation related to piracetam, it is important to clarify that piracetam is not a classic stimulant like amphetamine. The term "overstimulation" in the context of piracetam is not well-defined and is more speculative, as piracetam does not typically cause traditional stimulant-related side effects. Piracetam's primary action is thought to involve improving cognition and neuronal function. This can result in increased mental alertness, memory improvement, and potentially better concentration. In some people, particularly those sensitive to the effects of the drug or at higher doses, this could potentially lead to symptoms like anxiety, irritability, agitation, insomnia, or even headache. These are generally mild and reversible upon discontinuation of the drug or dose reduction. However, regarding the context of NTBI-induced mitochondriopathy, there is no established link between piracetam and exacerbation of symptoms directly related to mitochondrial dysfunction. As previously stated, some evidence suggests that piracetam might actually improve mitochondrial function and cellular energetics. Nevertheless, in the context of a complex medical condition, any medication can potentially have unpredictable effects. For example, in a patient with a severe mitochondrial disease where there's significant cardiac or neurological involvement, any change in physiological state - even one that might be beneficial under normal circumstances - could theoretically have unintended effects. But again, this is largely speculative and not something that has been specifically reported with piracetam. So, while some potential for piracetam to trigger "overstimulation" exists, it is still considered generally well-tolerated with relatively few side effects in most individuals. As with any medication, its use in complex medical conditions should be carefully considered, with close monitoring for any potential adverse effects.

Conclusion

A homozygous HFE gene H63D mutation can cause H63D syndrome, which might progress to (severe) mitochondriopathy if inadequately treated, sometimes even despite adequate treatment. The latter is a worst case scenario, as the disease process becomes so complex that there is less and less room for safe treatment approaches. A treatment experiment with piracetam is worth a try, but far from a cure.

Conflicts of interest

Nothing to declare.

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