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Research Article

The Role of Iron Homeostasis in Alzheimer's Disease

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Abstract

Background

Alzheimer's Disease (AD) is characterized by deposition of amyloid plaques of amyloid-β chelating peptide with transition metal ions (Cu^{2+}, Zn^{2+} and Fe^{3+}). The binding of Cu^{2+} and Fe^{3+} leads to toxic chemical reactions; a change in the oxidation of two metals, that leads to H_2O_2 production in the presence of transition metals and finally gives toxic free OH· radicals.

Methods

58 Alzheimer's disease patients were included in this study. They were evaluated for serum iron, copper, selenium, zinc and hepcidin levels. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) were measured as oxidative stress markers. Hepcidin, SOD and GPX were measured by ELISA methods. Serum Fe, Cu, Se and Zn were quantified by AAS. The results form AD patients were compared to age and gender matched healthy controls. We used Pearson's correlation and Student's paired t-test for statistical analysis of established results.

Results

We found statistically significant elevated serum iron, copper and zinc results in AD patients (41.4 µmol/l, 37.8 µmol/l, and 39.9 µmol/l) compared to control group (20.6 µmol/l, 19.1 µmol/l, and 14.8 µmol/l); $P<0.01$. Plasma selenium levels were decreased in AD patients (114.8 nmol/L) compared to healthy controls (629.6 nmol/L); $P<0.005$. Hepcidin concentrations were increased in AD cases (64.9 µg/l) compared to controls (22.3 µg/l); $P<0.001$. SOD and GPX levels were decreased in Alzheimer's disease (8.1 µg/ml, and 10.5 pg/mL) compared to normal values in healthy controls (20.4 µg/ml, and 37.4 pg/mL); $P<0.001$.

Conclusions

The expected contribution from our study is practical introduction of quantification of serum hepcidin as a potential marker for early diagnosis of impaired iron homeostasis, leading trace element in the pathogenesis of neurodegenerative diseases.

Keywords: Alzheimer's disease; Copper; Hepcidin; Iron homeostasis; Neurodegenerative diseases; Zinc

Abbreviations

AAS: Atomic Absorption Spectrometry
AD: Alzheimer's Disease
ALS: Amyotrophic Lateral Sclerosis
Aβ: Amyloid-β
BBB: Blood-Brain Barrier
Cu: Copper
CBC: Complete Blood Count
CHr: Concentration of Hemoglobin in Reticulocytes
DMT1: Divalent Metal Transporter 1
ELISA: Enzyme Linked Immunosorbent Assay
Fe: Iron
GPX: Glutathione Peroxidase
HD: Huntington's Disease
HIF-1: Hypoxia Inducible Factor 1
HIF2α: Hypoxia Inducible Factor 2α
PD: Parkinson's Disease
RNS: Reactive Nitrogen Species
ROS: Reactive Oxygen Species
Se: Selenium
SOD: Superoxide Dismutase
TIBC: Total Iron Binding Capacity
Zn: Zinc

Introduction

Oxygen is needed for energy metabolism and normal function of most eukaryotic organisms. It is partially reduced in the respiratory chain of low superoxide free basic radical, which can be converted into other forms of Reactive Oxygen Species (ROS). Cellular metabolism could generate other free radicals from nitrogen classified in the family of Reactive Nitrogen Species (RNS). ROS and RNS in physiological concentrations mediates a variety of normal functions, such as signal transduction regulation, induction of mitogenic response, even moderates the defense against infectious agents and others.

ROS are balanced with antioxidant systems to maintain a constant level in the body. These antioxidant systems are enzymatic and non-enzymatic. Violation of the balance of overproduction of ROS and/or reduction of antioxidants is called oxidative stress. Under these conditions, the large amount of free radicals may pass through the plasma membrane, damages the cell membrane through lipid peroxidation, and modifies the signal and structural proteins, resulting in

oxidation of the RNA/DNA, transcription termination, and occurrence of gene mutation.

The clearest aging effect in neuronal degeneration usually is associated with impaired trace elements metabolism -Cu, Fe and Zn. The aging brain becomes more susceptible to degenerative processes [1].

Neurodegenerative diseases are conditions in which the nervous system progressively and irreversibly deteriorates. Neurodegenerative diseases are often with late manifestation of disorders, including Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease (HD) and Amyotrophic Lateral Sclerosis (ALS).

AD depends on age; its a chronic neurodegenerative disease, which leads to dementia among the elderly. It is well known that AD is characterized by amyloid plaques deposition with transition metal ions (Cu^{2+}, Zn^{2+} and Fe^{3+}) [2].

Indirect mechanisms exist by which Hypoxia-Inducible Factor 1 (HIF-1) regulates hepcidin expression. Increased HIF activity is connected to secession of hemojuvelin from matriptase-2, which down regulates hepcidin expression [3]. Systematic iron regulation from hepcidin-ferroportin axis collaborates with control mechanisms on enterocytes cellular level. In mouse models deficiency of duodenal Hypoxia-Inducible Factor 2α (HIF2α) is observed decreased expression not only for ferroportin, but for Divalent Metal Transporter 1 (DMT1), which is the main iron exporter and is situated on apical surface of duodenal enterocytes. It is unable to place absorption, even if hepcidin secretion is decreased [4]. This might bring to conclusion, that in case of hypoxia, HIF2α expression leads to iron absorption from intestinal bowels through increased DMT1 and ferroportin activity. Therefore HIF2α is able to change iron transport, despite of hepcidin-ferroportin axis regulation.

Oxidative stress plays an important role in neuronal damage caused by cerebral ischemia. It was found that free iron is significantly increased during ischemia and is responsible for the oxidative damage of the brain. A number of mechanisms are involved in brain injury by oxidative stress [5,6]. Iron plays a critical role in neuronal damage caused by oxidative stress. Increased levels of Hypoxia Inducible Factor 1 (HIF-1) is an expression of high hepcidin secretion [7,8].

Methods

58 patients with Alzheimer's disease were included. Their results were compared to age and gender matched healthy controls. Alzheimer's disease patients were monitored in "Aleksandrovska" hospital, Clinic of Neurology.

Atomic Absorption Spectrometry (AAS- Perkin Elmer) was used for quantification of serum iron and Total Iron Binding Capacity (TIBC), serum Cu and Zn, and plasma Se. Serum ferritin levels were quantified by CLIA method (Immulyte 2000, by Siemens Healthcare). CBC, including hemoglobin concentration in reticulocytes (CHr) was quantified on ADVIA 2120 (provided by Siemens Healthcare). All chemical laboratory parameters were specified on Dimension RxL MAX (Siemens Healthcare Diagnostics). Included groups were evaluated for serum iron, copper, selenium, zinc and hepcidin levels. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) were measured as oxidative stress markers. Hepcidin, SOD and GPX were measured by ELISA methods (on VersaMax ELISA reader). Paired Student's t-test and Pearson's correlation were used for statistical analysis. Enrolled patients signed written informed consent according to the Declaration of Helsinki (Directive 2001/20/EC).

Results

Age distribution of patients in the different included groups is shown in table 1.

Group	Male	Age
Alzheimer's disease	28 (48.3%)	74.1 ± 8.6
Control group	28 (48.3%)	73.9 ± 7.1

Table 1: Age distribution of patients in included groups.

Established parameters from CBC and laboratory assessment are presented in table 2.

Parameter	AD			Control group		
	Mean	Min	Max	Mean	Min	Max
Hb (g/L)	139.5	118.0	161.0	141.8	121.9	161.7
RBC (*10^{12}/L)	4.01	3.21	4.81	4.75	4.05	5.45
CHr (pg)	23.7	21.1	26.3	31.9	29.1	34.7
Creatinine (μmol/L)	70.9	65.8	76.0	75.6	70.3	80.9
Iron (μmol/L)	41.4	37.9	44.9	20.6	18.4	22.8
TIBC (μmol/L)	75.6	71.5	79.7	63.8	60.9	66.7
Ferritin (ng/mL)	254.8	218.3	291.3	91.5	81.4	101.6
Glucose (mmol/L)	4.69	3.59	5.79	4.75	3.55	5.95
TRSF (g/L)	2.45	2.05	2.85	2.85	2.55	3.15
Zn (μmol/L)	39.9	38.3	41.5	14.8	14.0	15.6
Cu (μmol/L)	37.8	25.9	39.7	19.1	18.2	20.0
Se (nmol/L)	114.8	101.3	128.3	629.6	597.5	661.7
SOD (μg/mL)	8.1	6.9	9.3	20.4	17.9	22.9
GPX (pg/mL)	10.5	9.6	11.4	37.4	35.3	39.5

Table 2: Established parameters from CBC and laboratory assessment of included groups (presented as mean value and range).

*Hb - Hemoglobin, RBC - Red Blood Cells, CHr - Concentration of Hemoglobin in Reticulocytes, TIBC - Total Iron Bonding Capacity, TRSF - Transferrin, Zn - Zinc, Cu - Copper, Se - Selenium, SOD - Superoxide Dismutase, GPX - Glutathione Peroxidase

Serum iron and TIBC concentrations were increased in AD patients (41.4 μmol/L, and 75.6 μmol/L), compared to healthy controls (20.6 μmol/L, and 63.8 μmol/L); *P*<0.005. Plasma Se concentrations were decreased in AD (114.8 nmol/L) collate to control group (629.6 nmol/L); *P*<0.001. As an evidence of oxidative stress, SOD and GPX in alzheimer's disease cases were decreased (8.1 μg/L, and 10.5 pg/mL) in comparison to healthy controls (20.4 μg/L, and 37.4 pg/mL); *P*<0.005. We establish increased serum Zn and Cu levels in AD patients (39.9 μmol/L, and 37.8 μmol/L); in healthy controls they were 14.8 and 19.1 μmol/L; *P*<0.001. Ferritin as a sign of elevated iron showed elevated concentrations in neurodegenerative disease (254.8 ng/mL), compared to healthy controls (91.5 ng/mL); *P*<0.005.

We found statistically different results in included groups for quantified serum hepcidin levels. The results obtained are presented in figure 1.

SOD and GPX levels statistically significant differs in AD patients and control group (P<0.005) (Figure 2 and Figure 3).

Serum hepcidin levels statistically differ between Alzheimer's disease patients and healthy controls - 114.8 ± 10.1 μg/L to 22.3 ± 1.2 μg/L; *P*<0.001.

We found a significant correlation between serum iron levels and hepcidin concentration in included groups: patients with AD
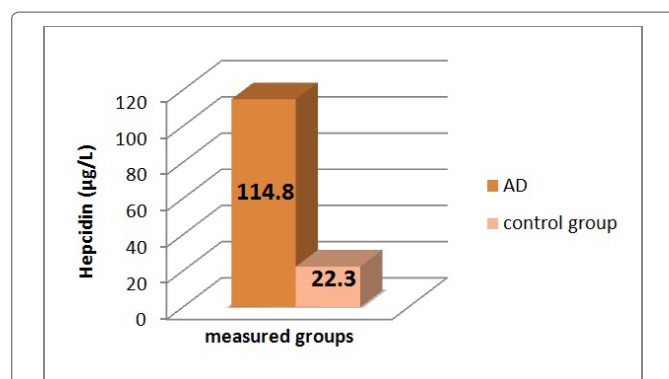
Figure 1: Serum hepcidin concentrations (in µg/L) in the different groups of patients included in this study.
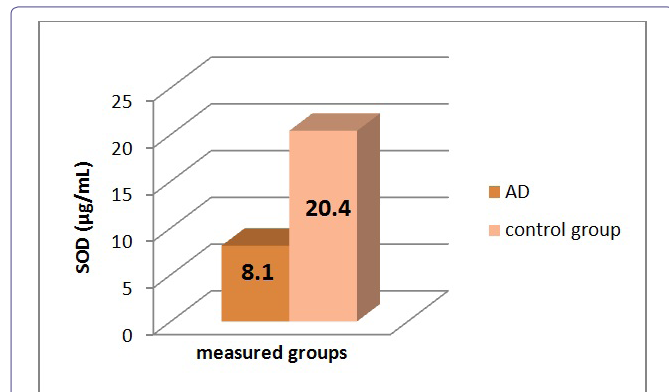
*AD - Alzheimer's Disease

Figure 2: SOD levels (in µg/mL) in the different groups of patients included in this study.

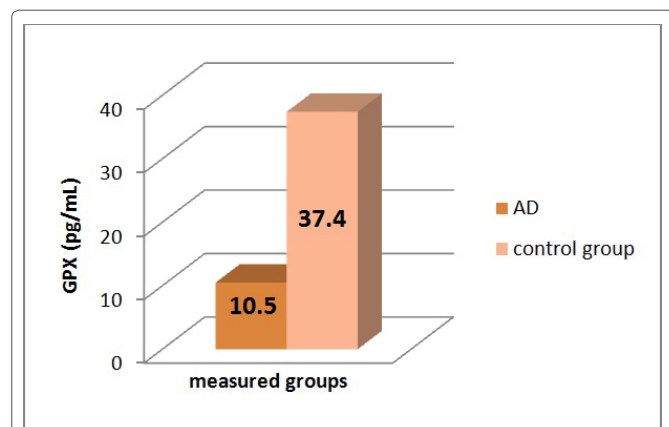*AD - Alzheimer's Disease, SOD - Superoxide Dismutase

Figure 3: GPX levels (in pg/mL) in the different groups of patients included in this study.

*AD - Alzheimer's Disease, GPX - Glutathione Peroxidase

(r=0.698, $P<0.001$), and in control group (r=0.423, $P<0.001$). Ferritin levels correlated to quantified serum hepcidin in AD patients (r=0.544, $P<0.005$), and in control group (r=0.491, $P<0.01$). Plasma levels of Se correlated to hepcidin in AD cases (r=-0.781, $P<0.005$). Serum Zn and Cu levels correlated to hepcidin concentration in neurodegenerative disease (r=0.771 and r=0.698, $P<0.005$). SOD and GPX correlated to hepcidin in patients with Alzheimer's disease (r=-0.825 and r=-0.865, $P<0.005$).

Discussion

Balance between production and elimination of ROS is essential for maintaining its physiological level and the normal functions of the body. ROS are generated at different locations in the cell, especially in mitochondria. There enzymatic and non-enzymatic systems maintain constant levels of ROS. At low levels of ROS, the body cannot perform certain physiological functions. Conversely, high levels of ROS, leading to functioning in conditions of oxidative stress.

In oxidative stress, high ROS levels change all bio-macromolecules, causing lipid peroxidation, impaired transcription of RNA and DNA damage with the occurrence of mutations. The role of ROS in the oxidation of proteins is the basis for neurodegenerative diseases. ROS mediate and/or cause neurotoxicity. Free radicals pass through the plasma membrane and lead to cell death. ROS leads to the aging of the organism.

The most striking effect of aging can be described in neurodegenerative diseases associated with disturbed metabolism of microelements. Upon aging in the brain was observed accumulation of redox-metals (copper, iron and zinc). This occurs because of increased concentration of metals over passing Blood-Brain Barrier (BBB) in connectivity of neuronal medium with blood vessels. This makes the brain more likely to develop neurodegeneration [9].

AD is characterized by deposition of amyloid plaques of Amyloid-β chelating peptide (Aβ) with transition metal ions (Cu^{2+}, Zn^{2+} and Fe^{3+}). The binding of Cu^{2+} and Fe^{3+} leads to toxic chemical reactions, a change in the oxidation of two metals, the production of H_2O_2 in the presence of transition metals and finally gives toxic free OH radicals [2].

Some studies demonstrates role of oxidatively modified lipids in AD pathogenesis [10]. Lipid peroxidation mediated by ROS, generates reactive carbonyl ingredients, which can react with cellular proteins, lipids, and DNA leading to DNA damage. In vitro conditions, authors represents that the copper and iron ions presence catalyzes the formation of hydrogenperoxide [12]. More in vitro models shows that chelation of copper and iron inhibits ROS formation. The role of zinc in AD etiology is impressive - zinc inhibits Aβ-induced toxicity [11,12]. Additionally, zinc is connected to copper in alzheimer's disease. While low zinc levels protects against Aβ-toxicity, its excess might lead to neuronaldeath. Under normal physiological conditions a sensitive balance exists between zinc, copper and Aβ-metabolism [13].

Neurodegenerative diseases associated with oxidative stress are not only caused by abnormal metabolism of trace elements, but there are genetic evidence suggesting that those associated with certain genetic mutations are more likely to develop nerve pathology compared to those with normal genetic profile. Patients with mutations in the HFE gene (for hemochromatosis) are more likely to develop oxidative stress and iron overload, leading to neuropathology [14,15].

Hepcidin is a key regulatory protein that controls the intestinal iron absorption and its distribution in the body [16]. It is a peptide hormone which is synthesized in the liver in response to such signals, including the levels of iron in the body. Hepcidin functions by binding and initialization degradation molecule ferroportin, the only known iron exporter. Ferroportin is located in the transmembrane duodenal enterocytes, macrophages and hepatocytes. It regulates the transfer of iron in plasma cell by these cells [17,18]. High levels of iron in the circulation stimulate the synthesis of hepcidin in the liver. Increased hepcidin reduces intestinal iron absorption and blocking exports by

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tissue depots-thus avoiding over-accumulation of iron in the body. On the other hand, decreased iron levels inhibit the synthesis of hepcidin and stimulate the absorption of iron in duodenal enterocytes and his release from tissue depots. Some researchers found decreased brain hepcidin deposition [19]. We quantified iron homeostasis regulator in serum.

Elevated hepcidin leads to decreased transferrin bound iron [20]. This is suggested to cause age-related anaemia [21].

Conclusion

Our study has some limits. The most important is the number of included patients. Still, we think our study contributes in practical introduction of quantification of serum hepcidin as a potential marker for early diagnosis of impaired iron homeostasis, leading trace element in the pathogenesis of neurodegenerative diseases. Increased serum hepcidin levels in combination with early manifestations of impaired information processing might be a useful guideline for focusing on early Alzheimer's disease manifestations.

Acknowledgment

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