Trace Elements and Antioxidant in Multiple Sclerosis

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ABSTRACT

Background: No single environmental exposure has been consistently identified as a causal factor in MS. It has been suggested that trace elements and oxidative stress could be implicated in the pathogenesis of multiple sclerosis (MS).

Aim of Study: The present work aimed to estimate serum levels of some trace elements; copper (Cu), zinc (Zn); manganese (Mn), and glutathione peroxidase (GSH-PX) as a marker of antioxidant in multiple sclerosis patients in comparison to a control group and to verify whether these trace elements level related to clinical data and to correlate them with each other.

Patients and Methods: Forty multiple sclerosis patients [10 males (25%) and 30 females (75%)]. Their age ranged from 17 to 44 years with a mean age 30.53±8.14 years were evaluated clinically using EDSS. In addition 15 age and sex matched healthy volunteers as a control group were recruited. The analysis of trace elements copper, zinc and manganese were performed by atomic absorption spectrophotometry. The oxidative stress in the blood were assessed by performing a multiparameter analysis of glutathione peroxidase (GSH-PX) by GC-MS; UBI.

Results: Serum levels (mean ± SD) of MANGANESE, (GSH-PX), ZINC and COPPER were 4.44±0.93, 21.49±2.52, 5.32±0.43 and 830.2±100.04 micromol/l respectively. Mean of glutathione peroxidase and copper were statistically significantly lower in patients than in the control group whereas the mean manganese and zinc level were statistically significantly higher. Mean of zinc level was statistically significantly lower in female patients than in males. A statistically significant negative correlation was found between degree of disability as measured by EDSS scores and manganese level. Mean of copper level was significantly reduced in SPMS than the RRMS and PPMS. There was a significant correlation between serum levels of (GSH-PX) and trace elements; positive with copper and negative with Zn and Mn. There was a significant negative correlation between copper and Mn.

Conclusions: Patients with MS show altered trace elements and antioxidant. There is a significant correlation between investigated trace element and patients’ gender and degree of disability. There is also a significant correlation between investigated trace element with each others and antioxidants.

INTRODUCTION

Trace element neurotoxicity and oxidative stress have been involved as an etiologic factor for multiple sclerosis (MS)¹. Zinc (Zn), copper (Cu) and manganese (Mn) are essential trace elements that play a major role in various metabolic pathways². Nutritional deficiencies of trace elements and/or accumulations of toxic heavy metals have been cited as important contributing factors in MS³. Several controlled studies showed that MS patients consistently exhibit low levels of zinc⁴. Zinc plays an extremely important role in immune function, inhibiting certain potentially damaging immune reactions of T lymphocytes implicated in the autoimmune degeneration seen in MS. Zinc also serves as an important antioxidant, with an ability to safeguard various cell membranes including myelin⁵.

Copper level is also commonly altered in MS; the body needs optimal amounts of copper for sustaining proper energy production, and for ensuring the integrity of the myelin sheaths⁶. Manganese is important for the metabolism of nitric oxide, and for many enzymes⁷. Manganese is also important in the development, structure and stability of myelin its deficiency is associated with increased inflammation and peroxynitrite damage in multiple sclerosis⁸.
Accumulating data indicate that oxidative stress (OS) plays a major role in the pathogenesis of multiple sclerosis (MS). Reactive oxygen species (ROS) have been implicated as mediators of demyelination and axonal damage resulting in cell death by necrosis or apoptosis. It is postulated that brain and nervous system cells are prone to oxidative damage because of their relatively low content of antioxidants, especially enzymatic ones. In addition, weakened cellular antioxidant defense systems in the central nervous system (CNS) in MS, and its vulnerability to ROS effects may increase damage. Glutathione peroxidase, one of the major antioxidants in the human brain, has been found to have decreased activity in patients suffering from multiple sclerosis. (GSH-PX) activity in the erythrocytes was found to be lower in the MS patients. The possibility of counteracting MS by antioxidant administration plus an appropriate diet might represent a promising way of inhibiting the progression of the disease. Treatment with antioxidants might theoretically prevent MS and may prevent propagation of tissue damage and improve both survival and neurological outcome in MS patients.

Recently several studies have reported that Zn, Mn, Cu and (GSH-PX) were statistically significantly lower in MS patients than in the control group. However, other study has been found them to be higher in central nervous system tissue in MS patients, especially in white matter.

This study aimed to estimate serum levels of some trace elements; manganese (Mn), copper (Cu), zinc (Zn); and glutathione peroxidase (GSH-PX) in multiple sclerosis patients compared to a group of age and sex matched healthy subjects and to verify whether these trace element level related to clinical data and to correlate them with each other.

**PATIENTS AND METHODS**

**Patients:**
This study included 40 multiple sclerosis patients [10 males (25%) and 30 females (75%)]. recruited from the Neurology outpatient clinic of Kasr El-Aini University hospitals and Fayoum University or admitted to the Neurology Department, Cairo University. Their ages ranged from 17 to 44 years with a mean age of 30.53±8.14 years.

**Inclusion criteria**
Definite MS: All patients included in the study were diagnosed as having clinically definite multiple sclerosis according to Poser et al. criteria or having MRI supported definite MS according to Paty et al. criteria. Patients were diagnosed as having Relapsing Remitting MS (RRMS), Primary progressive MS (PPMS) or Secondary progressive MS (SPMS) according to Lublin et al. criteria.

Normal routine laboratory investigations (CBC, blood glucose level, renal and liver functions)

**Exclusion criteria**
Patients with any associated medical or neurological diseases were excluded from this study.

**Control group:**
In addition 15 age and sex matched healthy volunteers were recruited.

**Methods:**

**Clinical evaluation**
Patients were evaluated clinically using.

**Thorough history taking and detailed neurological evaluation**
Thorough history taking and detailed neurological evaluation were done using Kasr El Aini neurology department MS sheet paying attention to age of onset, duration of illness, precipitating factors, MS course and whether in activity or relapse in RRMS, in progression or plateau phase in secondary progressive MS.

**Expanded disability status scale (EDSS)**
It is a functional scale that objectively determines and quantifies the degree of disability and monitor disease progression and drug response. EDSS quantifies disability in eight functional systems, pyramidal, cerebellar,
brainstem, sensory, bowel, and bladder, visual, mental and other functions. It is 20 grades ranging from 0, 0.5, 1, and 1.5 till 10. EDSS score of up to 4.5 refer to people with MS who are fully ambulant. EDSS score of 5.0 or more are defined by impairment to ambulation.

Neuroradiological studies: MRI brain and or spinal cord with contrast were done for all patients on a 1.5 Tesla Phillips intra® scanner.

Biochemical analysis
The analysis of trace elements copper, zinc and manganese were performed by atomic absorption spectrophotometry using the Perkin-Elmer atomic absorption spectrophotometer (model 2380). Furnace graphite HGA-300 was used to detect minute amount of this trace elements and an acetylene flame was used to measure the Mn level according to Papageorgiou et al.19. The antioxidants, glutathione peroxidase (GSH - PX) activity was measured spectrophotometrically according to Plagia and Valentiene20 using Wak-Chemie kit (Germany).

Statistical analysis
Software package SPSS version 13 was used for statistical analysis. Quantitative data were presented as means and standard deviations. Qualitative data were presented as percentages or ranges and large numbers as means and standard deviations. Pearson Qui square was used for comparisons. P<0.05 was considered statistically significant. P<0.001 was considered highly statistically significant. Analysis of variance (ANOVA) was used for comparisons of > 2 variance. Correlation was used to study association between variables.

RESULTS

Clinical results
This study included 40 patients with multiple sclerosis. Their ages ranged from 17 to 44 years with a mean age 30.53±8.14 years. They were 10 males (25%) and 30 females (75%). This study also included 15 healthy age and sex matched volunteers as a control group.

Course of Multiple sclerosis
In this study 23 patients (60%) of the patients had secondary progressive MS, 14 patients (33%) had relapsing remitting MS and 3 patients (7%) had primary progressive MS as shown in Figure (1).

No one of our patient was in a stage of activity (exacerbation), in the secondary progressive type they were in a stage of plateau and in the relapsing remitting type they were in remission.

The duration of MS illness
The duration of MS illness ranged from 1 to 11 years with a mean 4.77±2.79 years.

Expanded Disability status Scale
Expanded Disability Scales in the patients ranged from 2 to 8.5. EDSS ranged from 2-4.5, 3-3.5 and 3.5-8.5 in relapsing remitting, primary progressive and secondary progressive Ms respectively.

Comparison between EDSS and clinical data
EDSS was statistically significantly higher in secondary progressive MS type. No significant relation was found between EDSS and sex, table (1).

No significant correlation was found between EDSS and age (r 0.085, P 0.312) or EDSS and duration of illness (r 0.188, P 0.429).

Biochemical results
Mean of measured trace elements in the patients were as follow Copper 830.2±100.04, Zinc 5.32±0.4, (GSH - PX) 21.49±2.52, Manganese 4.4±0.9, as presented in figure (2).

Comparison between mean of trace elements in patients and control groups
Mean of glutathione peroxidase and copper were lower in patients than in the control group.
whereas the mean of manganese and zinc level were higher and these differences were highly statistically significant, table (2) and Figure (3).

Mean of trace elements in patients and control groups is illustrated in Figure (3).

**Comparison between mean of trace elements and patients gender**

Mean of zinc level was statistically significantly lower in female than in male patients. No significant differences were found between other trace elements and patients' gender, table (3).

**Comparison between the mean of trace elements in different types of MS course**

The mean of copper level was statistically significant lower in secondary progressive MS in comparison to the other two types (RRMS & PPMS). Whereas no statistically significant difference was found with other trace elements, table (4).

**Correlations between levels of trace elements and disease severity and disability as measured by EDSS score and duration of illness**

Highly statistically significant negative correlation was found between degrees of disability as measured by EDSS score and manganese level. Whereas, no statistically significant correlation was found between EDSS scores and other trace elements. No statistically significant correlation was found between disease duration and trace elements levels, table (5).

**Correlations between Glutathione peroxidase and trace elements**

Glutathione peroxidase (GSH - PX) as a marker of antioxidant in multiple sclerosis patients was found to be significantly correlated with trace elements; positively with copper (Cu) and negatively with zinc (Zn); and manganese (Mn), table (6) and figure (4).

**Correlation between copper and other trace elements**

Copper was found to be significantly negatively correlated with manganese (r=-0.428, P 0.001) figure (5). No significant correlation was found with zinc (r =-0.180, P 0.198); No significant correlation was found between zinc and manganese (r=0.084, P 0.548).

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**Fig. (1): Course of MS in the patients.**
Table 1. Comparison between EDSS and clinical data.

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>EDSS mean</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>5.37±1.21</td>
<td>0.9</td>
</tr>
<tr>
<td>Females</td>
<td>4.66±1.61</td>
<td></td>
</tr>
<tr>
<td><strong>MS Course</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing remitting MS</td>
<td>3.44±0.80</td>
<td>0.000</td>
</tr>
<tr>
<td>Primary progressive</td>
<td>3.25±0.35</td>
<td></td>
</tr>
<tr>
<td>Secondary progressive</td>
<td>5.93±1.41</td>
<td></td>
</tr>
</tbody>
</table>

Fig. (2): Means of trace elements in patients group.

Table 2. Comparison between mean of trace elements in patients and control groups.

<table>
<thead>
<tr>
<th>Biochemical results</th>
<th>Patients</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(GSH - PX)</td>
<td>21.49±2.52</td>
<td>31.51±1.50</td>
<td>0.000</td>
</tr>
<tr>
<td>COPPER</td>
<td>830.2±100.04</td>
<td>1077.74±111.41</td>
<td>0.000</td>
</tr>
<tr>
<td>MANGANESE</td>
<td>4.4±0.9</td>
<td>2.88±0.6</td>
<td>0.000</td>
</tr>
<tr>
<td>ZINC</td>
<td>5.3±0.4</td>
<td>5.05±0.13</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 3. Comparison between mean of trace elements and patients gender.

<table>
<thead>
<tr>
<th>Biochemical Results</th>
<th>Females</th>
<th>Males</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MANGANESE</td>
<td>4.44±0.97</td>
<td>4.43±0.84</td>
<td>0.9</td>
</tr>
<tr>
<td>ZINC</td>
<td>5.21±0.34</td>
<td>5.66±0.52</td>
<td>0.02</td>
</tr>
<tr>
<td>(GSH - PX)</td>
<td>21.74±2.81</td>
<td>20.74±1.09</td>
<td>0.1</td>
</tr>
<tr>
<td>COPPER</td>
<td>853.22±97.66</td>
<td>830.41±88.55</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 4. Comparison between MS course and Biochemical results.

<table>
<thead>
<tr>
<th>Biochemical results</th>
<th>Relapsing Remitting</th>
<th>Primary Progressive</th>
<th>Secondary Progressive</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPPER</td>
<td>903.35±55.72</td>
<td>807.02±120.8</td>
<td>802.07±103.8</td>
<td>0.04</td>
</tr>
<tr>
<td>MANGANESE</td>
<td>4.96±0.89</td>
<td>5.05±1.82</td>
<td>4.11±0.88</td>
<td>0.08</td>
</tr>
<tr>
<td>(GSH - PX)</td>
<td>40.98±1.25</td>
<td>20.63±0.41</td>
<td>21.76±2.57</td>
<td>0.6</td>
</tr>
<tr>
<td>ZINC</td>
<td>5.39±0.35</td>
<td>5±0.01</td>
<td>5.3±0.37</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 5. Correlation between MS severity, duration of illness and biochemical results.

<table>
<thead>
<tr>
<th>Biochemical results</th>
<th>EDSS Scores</th>
<th>Disease duration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation Coefficient</td>
<td>P Value</td>
</tr>
<tr>
<td>MANGANESE</td>
<td>-0.526</td>
<td>0.006</td>
</tr>
<tr>
<td>(GSH - PX)</td>
<td>0.239</td>
<td>0.24</td>
</tr>
<tr>
<td>Copper</td>
<td>-0.257</td>
<td>0.2</td>
</tr>
<tr>
<td>ZINC</td>
<td>-0.281</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 6. Correlation between (GSH - PX) and trace elements.

<table>
<thead>
<tr>
<th>Trace elements</th>
<th>(GSH - PX)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation Coefficient</td>
</tr>
<tr>
<td>MANGANESE</td>
<td>-0.529</td>
</tr>
<tr>
<td>Copper</td>
<td>0.678</td>
</tr>
<tr>
<td>ZINC</td>
<td>-0.321</td>
</tr>
</tbody>
</table>

Fig. (4): Illustrates the statistically positive correlation between GSH - PX and Copper levels.
DISCUSSION

Although some studies suggested a link between exposure to trace elements and development of multiple sclerosis (MS), clear information on their role in the etiology of MS is still lacking. One of the postulated links previous studies reported is that, trace elements (zinc, copper, magnesium, etc) act as co-factors enable the liver to detoxify natural toxins in different foods. Poor detoxification means that these toxins will circulate freely in the body. Some inevitably find their way into the brain. These toxins bind with the fatty myelin sheaths. Immune system starts to attack it with consequent inflammation and destruction of the myelin sheath. Also the trace elements may affect antioxidant defense system as essential trace elements copper, zinc and manganese are important parts of antioxidant enzymes as superoxide dismutase, glutathione peroxidase and important parts of transport protein with antioxidant properties as ceruloplasmin. Nutritional deficiencies and/or accumulations of key minerals and toxic heavy metals have been cited as important contributing factors that can adversely impact the health of patients with MS. Numerous studies of patients with multiple sclerosis have shown increased free radical activity, and/or deficiencies of important antioxidant enzymes compared with healthy controls.

In the present work in agreement with Syburra et al. and Rukgauer et al., mean of glutathione peroxidase was statistically significantly lower in MS patients compared to the
control (P 0.000). Astrocytes play a central role in the antioxidant defense of the brain via maintaining high intracellular concentrations of certain antioxidants; glutathione peroxidase (GSH - PX) is one of the major antioxidants used by astrocytes making these cells resistant to oxidative stress. Some studies suggest that (GSH - PX) protects cells from hypoxic injury by direct inhibition of neutral SMases activity and ceramide formation. This protection appears to be lost in MS patients as (GSH - PX) is decreased in plaques in MS.

In this study in agreement with Johnson et al. and Wikstrom et al. mean of copper was statistically significantly lower (P 0.000) in MS patients compared to the control. Fat malabsorption was reported in multiple sclerosis. There is a possibility that malabsorption of the copper causes its low serum concentrations. This decrease in copper concentrations in MS patients could lead to deficient copper-containing enzymes as cytochrome oxidase. It also results in deficient Cu Zn Superoxide dismutase (CuZnSOD), which subsequent increase levels of superoxide; increasing oxidative stress. Previous studies found reduced activity of superoxide dismutase and glutathione peroxidase, powerful antioxidant enzymes in patients with MS, making them more vulnerable to oxidative stress than healthy controls. Moreover, the present work revealed that, the mean of copper level was significantly lower in secondary progressive MS patients, in whom there is a higher degree of disability. Whereas no statistically significant difference was found with other trace elements.

In this study in accordance to Williams et al. and Palm et al. mean of zinc level was statistically significantly higher in patients than in the control group (P 0.001). On the contrary, Melo did not find significant differences between zinc levels in MS and control patients. Another study has shown that zinc level is significantly elevated in patients with multiple sclerosis (MS) in between attacks and that this level was dramatically decreased during a clinically documented exacerbation of MS. It has been suggested that mechanisms which govern cellular availability, compartmentalization of Zn, or the binding of Zn to cell surface membranes may be altered in patients with MS, according to disease activity. It is important to mention here that no one of our patients was in a stage of activity they were either in remission in RRMS or a stage of plateau in the SPMS or PPMS which might explain the higher level of zinc reported in our patients.

It has been found that high level content of Mn subscribed to the formation of cerebral vascular deformation and sclerosis. In the present work in accordance to Melo et al. Zapakniuk et al. mean of manganese level was statistically significantly higher in patients than in the control group (P 0.000). Conversely Yanik et al. found that Manganese levels was significantly decreased in MS patients compared to the levels in the control group. The physiological basis for the differences in manganese concentrations between MS patients and controls is unknown, but could be related to alterations in the manganese-containing enzyme glutamine synthetase.

Whereas the present work revealed that the mean of manganese level was significantly higher in patients than in the control group, a statistically significant negative correlations was found between the degree of disability as measured by EDSS score and manganese level which signifies that with increasing degree of disability there's a decreasing level of manganese this could be explained by that the extra cellular Mn is required for NO release from the cell, so that a deficiency of these nutrients results in increased NO production in the cell and reduced release from the cell. The trapped NO combines with superoxide to form peroxinitrite, an extremely powerful free radical that leads to the myelin damage. The literature implicates excessive or inappropriate generation of nitric oxide (NO) in multiple sclerosis. Human astrocytes released abundant NO upon stimulation with the pro-inflammatory cytokine (IL) -1b. It is now well documented that NO and its toxic metabolite, peroxynitrite (ONOO−) can inhibit components of the mitochondrial respiratory chain. In this study no statistically significant correlation was
found between disease duration and mean of trace elements. This could be explained by the fact that it is not the matter of duration but number and severity of remissions that influence myelin damage in MS.

In this study in agreement with Palm et al.\textsuperscript{34} Mean of zinc level was statistically significantly lower in female than in male patients. This could be explained by the fact that females have an increased demand for zinc (Zn) and their rapidly decreasing production of melatonin results in impaired Zn absorption.

In this study in accordance to Rukgauer et al.\textsuperscript{25} no relation between concentration of Cu or Mn and sex could be established. In contrast Palm et al.\textsuperscript{34} reported that menstruating women have increased copper (Cu) absorption and half-life, so they tend to accumulate more Cu than males and often present with low manganese (Mn). Possibly this difference between our results and theirs could be attributed to the difference in patients age. Previous study had found that the manganese levels exhibited an age-dependent linear decrease and the copper concentrations exhibited the highest value in the age group of 6 to 10 years\textsuperscript{38}. In this study all patients were in adult age.

In our study no significant correlation was found between zinc and copper. In contrast Magalova et al.\textsuperscript{31} had found that Cu correlated significantly with Zn. The author explains this through impairment of Zn absorption by the high Cu level. This difference in results may be due to the fact that most of our patients were on steroids which might influence Cu and Zn homeostasis. As it has been suggested that in MS there is altered Cu and Zn homeostasis that may cause or result from the disease and is influenced by corticosteroid therapy\textsuperscript{1}.

In the present work, in accordance to Korpela et al.\textsuperscript{26} and Dudek et al.\textsuperscript{27} Glutathione peroxidase (GSH - PX) as a marker of antioxidant in multiple sclerosis patients was found to be significantly correlated with trace elements; positively with copper (r 0.678, P 0.000) and negatively with zinc (r -0.321, P 0.019) and manganese (r -0.529, P 0.000). These results demonstrated that Cu concentration can improve the trace element dependent antioxidative status. Another study had reported that the higher the concentrations of Cu, the higher the activities of SOD. The susceptibility of different brain cells to NO and ONOO− exposure may be dependent on factors such as the intracellular (GSH - PX) concentration\textsuperscript{32}.

**Conclusion**

There are trace element and antioxidants alterations in MS. These alterations correlated with MS severity and course. Lower levels of Cu was associated with a higher degree of disability and lower levels of (GSH - PX). There is a correlation between trace element and antioxidants.

**REFERENCES**


الملاحظات العربية

لم يتمكن العلماء حتى الآن من تحديد العوامل البيئية المسببة لمرض النصب المتكرر، ولكن، فقد تتراوح في المواد النادرة ومضادات الأكسدة من أسباب هذا المرض.

لذا تهدف هذه الدراسة إلى قياس نسبة "الحساس، والزنك، والمنجنيز، وجلوتاتيون بيراكسيدة " في مرضا النصب المتكرر، ومقارنتها في مجموعة ضابطة من الأصحاء، وتحديد العلاقة بين هذه المواد والحالات الإكلينيكية للمرضى وكذلك مقارنتها بعضها البعض.

اشتملت هذه الدراسة على 40 حالة من مرضا النصب المتكرر (10 حالات من الذكور، 30 حالة من الإناث)، تراوحت أعمارهم بين 17 ، 44 سنة، وكان متوسط العمر 30 سنة، بالإضافة إلى 15 من الأصحاء متماثلين مع المرضا في العمر والجنس كمجموعة ضابطة.

تم عمل مقياس الإعاقة لهم، وتحليل نسبة المواد النادرة باستخدام جهاز "ج س "، وأوضحت النتائج النسب التالية: نسبة الحساس في المرضى 830.2 نسبة الزنك في المرضى 5.3 نسبة المنجنيز 44 جلوتاتيون 21.4 وكانت نسبة المنجنيز العالي في المرضى عينها في المجموعة الضابطة، بينما كانت نسبة الحساس والجلوتاتيون أقل، وكانت فروقات ذات دلالة إحصائية، ووجد أن نسبة الزنك أقل في الإباط عن الذكور والفرق ذو دلالة إحصائية.

وكان هناك فرق ذو دلالة إحصائية بين نسبة المنجنيز وقياس الإعاقة في المرضى، بينما لم يكن هناك فروق ذات دلالة إحصائية مع نسبة النحاس ونوع المرض، بينما لم تكن هناك فروقات ذات دلالة إحصائية مع نسبة النحاس.

وكان هناك ارتباط طبيعي بين الجلوتاتيون والنحاس بينما كان هناك ارتباط عكسي بين الجلوتاتيون وكلا من الزنك والمنجنيز وارتباط عكسي بين النحاس والمنجنيز.

نتجه من هذه الدراسة وجود علاقة بين المواد النادرة ومضادات الأكسدة، ومرض النصب المتكرر، وكذلك علاقة بين المواد النادرة ومضادات الأكسدة.