ORIGINAL ARTICLE

# Increased levels of transition metals in breast cancer tissue

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## Abstract

**OBJECTIVES**: High levels of transition metals such as iron, nickel, chromium, copper, and lead are closely related to free radical generation, lipid peroxidation, formation of DNA strand breaks, and tumor growth in cellular systems. In order to determine the correlation to malignant growth in humans, we investigated the accumulation of heavy metals in 20 breast cancer biopsies and compared the findings to the levels found in 8 healthy biopsies.

**METHODS**: The concentration of transition metals in breast cancer and control biopsies was assessed by a standardized Atomic Absorption Spectrofotometry technique with acidic hydrolysis for sample preparation. Additionally, heavy metal analysis in control biopsies was also performed with an Inductive Coupled Plasma – Mass Spectroscopy technique. For statistical analysis of the results, the Mann-Whitney U Test was applied.

**RESULTS**: A highly significant accumulation of iron ($p<0.0001$), nickel ($p<0.00005$), chromium ($p<0.00005$), zinc ($p<0.00001$), cadmium ($p<0.005$), mercury ($p<0.005$), and lead ($p< 0.05$) was found in the cancer samples when compared to the control group. Copper and silver showed no significant differences to the control group, whereas tin, gold, and palladium were not detectable in any biopsies.

**CONCLUSIONS**: The data suggest that pathological accumulation of transition metals in breast tissue may be closely related to the malignant growth process.

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**Abbreviations & Units**

AAS: Atomic Absorption Spectrophotometry
EDDA: Ethylendiamine N,N'-diacetate
ICP-MS: Inductive Coupled Plasma – Mass Spectroscopy
MELISA®: Memory Lymphocyte Immuno Stimulation Assay
NTA: Nitrilotriacetic Acid

## Introduction

Reports in the last two decades closely relate the presence of transition metals like iron (Fe) or copper (Cu) to free radical generation via Fenton- and Haber-Weiss-reactions, ascorbate autoxidation, lipid peroxidation processes, and formation of DNA strand breaks [2, 12, 14, 19]. As published previously, lipid peroxidation-induced malondialdehyde-DNA adducts can accumulate and reach high levels in the breast tissue of women with breast cancer leading to endogenous DNA modifications [24]. Furthermore, ferric-ethylendiamine N,N'-diacetate (EDDA)- and nitrilotriacetic acid (NTA)-complexes were shown to induce free radicals and renal carcinomas in Wistar rats, demonstrating the key role of transition metals in the abnormal proliferation process [9, 16]. Since repeated mitochondrial and nuclear DNA mutations may lead to malignant growth, we investigated the heavy metal content in breast cancer biopsies and in healthy breast tissue biopsies.

## Material & Methods

Heavy metal analyses were performed on 20 frozen breast cancer biopsies and 8 healthy breast tissue samples supplied by the Institute of Pathophysiology and Oncology, Charles University, Prague, Czech Republic, and the Caritas Hospital St. Josef, Regensburg, Germany.

The concentrations of Fe, cadmium (Cd), lead (Pb), chromium (Cr), tin (Sn), nickel (Ni), Cu, mercury (Hg), silver (Ag), gold (Au), palladium (Pd), and zinc (Zn) in the biopsy materials were measured in the Spezialklinik Neukirchen, Germany, by a standardized furnace-atomic absorption spectrophotometry (AAS)-technique using a Perkin Elmer Sima 6000 AA-spectrophotometer and acidic hydrolysis as pulping procedure for sample preparation [17].

Additionally, heavy metal analysis in control biopsies was done using an inductive coupled plasma-mass spectroscopy (ICP-MS) technique in the Laboratory for Micro Trace Minerals, Hersbruck, Germany. Each analysis was performed three times. The final result per sample is the mean value of three determinations expressed in µg/kg. The Mann-Whitney U Test was used for statistical analysis of the results.

## Results

Data analysis showed a highly significant accumulation of Fe, Ni, Cr, Zn, Hg, Cd, and, to a lesser extent, of Pb in malignant breast tissue when compared to healthy breast tissue (Table 1).

Iron levels were dramatically increased in the breast cancer biopsies when compared to the control group (median: 53,174 µg/kg, range: 14,391–205,930 vs 10,937 µg/kg, range: 5,331–21,646) ($p<0.0001$).

A highly significant Ni accumulation was recorded in the patient biopsies (median: 995 µg/kg, range: 469–3,361). Control biopsies showed measurable levels (median: 21 µg/kg, range: 11–33), but at more than one order of magnitude lower ($p<0.00005$). Similar results were found for Cr when compared to the control group (median: 816 µg/kg, range: 313–5,978 vs 39 µg/kg, range: 19–119) ($p<0.00005$).

A surprisingly high accumulation of Zn was recorded in the cancer biopsies (median: 17,075 µg/kg, range: 1,326–97,895), the difference to the control group (median: 3,741 µg/kg, range: 2,548–9,339) again being highly significant ($p<0.001$).

Mercury was found moderately increased in 11 out of 20 cancer samples (median: 6.9 µg/kg, range: 1.8–45.9), a highly significant difference when compared to the control group (median: 2.1 µg/kg, range: 0.1–6.6) ($p<0.005$).

Increased Cd concentrations were found in 18 out of 20 cancer biopsies (median: 42 µg/kg, range: 9–551), the difference to the control group (median 16 µg/kg, range: 5–30) being highly significant ($p<0.005$).

Lead was also increased in 12 out of 20 tumor biopsies (median: 105 µg/kg, range: 9–976). The statistical difference to the control group (median: 64 µg/kg, range: 1–92) was still significant ($p<0.05$) (data not shown).

Surprisingly, lower Cu levels were found in 11 out of 20 patient biopsies (median: 919 µg/kg, range: 320–44,687), when compared to the control samples (median: 1,280 µg/kg, range: 261–3,049). Of the remaining nine cancer samples, seven showed increased values and two were in the normal range, documenting a different accumulation pattern possibly related to the tumor aetiology or growth stage. Taken together no significant difference was recorded between the cancer group and the controls ($p=0.65$) (data not shown).

Only four out of the 20 cancer samples, but none of the control biopsies, showed detectable levels of Ag (range: 34–91 µg/kg) (data not shown). Tin, Au, and Pd were not detectable in either cancer or control biopsies. When the content of heavy metals in control biopsies was analysed by two methods (AAS and ICP-MS) the values were not significantly different (data not shown).

## Discussion

To our knowledge, this is the first report describing a large accumulation of Fe and other transition metals like Ni, Cr, Zn, Cd, Hg, and Pb in the breast cancer tissue. These findings may have an implication for the pathogenesis of breast cancer. The etiology of human breast cancer is still controversial, although hormonal influences, toxic compounds, oxidative stress, and lipid peroxidation have been suggested to play a role in breast cancerogenesis.

**Table 1.** Heavy metal content in breast cancer (n = 20) and healthy breast tissue (n = 8) biopsies

| | Breast cancer biopsies | | | | | |
|---|---|---|---|---|---|---|
| **Patients** | **Fe µg/kg** | **Ni µg/kg** | **Cr µg/kg** | **Zn µg/kg** | **Hg µg/kg** | **Cd µg/kg** |
| 1 | 27,381 | 893 | 655 | 6,268 | 2.1 | 165 |
| 2 | 205,930 | 733 | 410 | 6,420 | 4.1 | 33 |
| 3 | 14,664 | 530 | 316 | 6,022 | 1.8 | 168 |
| 4 | 29,813 | 760 | 513 | 9,594 | 8.2 | 43 |
| 5 | 48,573 | 1,001 | 366 | 13,068 | 6.1 | 35 |
| 6 | 32,347 | 921 | 701 | 8,965 | 33.4 | 62 |
| 7 | 47,796 | 949 | 855 | 5,929 | 7.8 | 120 |
| 8 | 29,385 | 1,230 | 838 | 8,197 | 7.3 | 9 |
| 9 | 37,154 | 469 | 313 | 32,642 | 9.1 | 142 |
| 10 | 142,391 | 1,285 | 968 | 56,838 | 4.9 | 20 |
| 11 | 80,164 | 1,152 | 4,415 | 22,888 | 11.9 | 124 |
| 12 | 58,453 | 1,433 | 1,786 | 97,895 | 12.5 | 40 |
| 13 | 106,350 | 3,361 | 5,978 | 32,917 | 2.2 | 551 |
| 14 | 28,723 | 490 | 458 | 1,326 | 5.2 | 22 |
| 15 | 65,112 | 988 | 793 | 50,516 | 9.0 | 96 |
| 16 | 84,816 | 1,057 | 906 | 21,082 | 7.6 | 16 |
| 17 | 76,608 | 1,277 | 1,362 | 53,336 | 45.9 | 42 |
| 18 | 72,376 | 1,528 | 1,389 | 53,709 | 6.5 | 42 |
| 19 | 42,254 | 624 | 708 | 6,953 | 2.8 | 34 |
| 20 | 57,774 | 1,142 | 1,562 | 27,319 | 4.1 | 29 |
| Median | 53,174 | 995 | 816 | 17,075 | 6.9 | 42 |
| | **Healthy breast tissue biopsies** | | | | | |
| **Controls** | **Fe µg/kg** | **Ni µg/kg** | **Cr µg/kg** | **Zn µg/kg** | **Hg µg/kg** | **Cd µg/kg** |
| 1 | 5,331 | 32 | 29 | 2,548 | 2.5 | 6 |
| 2 | 11,448 | 11 | 19 | 3,509 | 6.6 | 8 |
| 3 | 21,646 | 19 | 36 | 3,973 | 2.3 | 23 |
| 4 | 11,424 | 32 | 119 | 2,940 | 1.9 | 5 |
| 5 | 10,138 | 33 | 70 | 4,032 | 2.5 | 8 |
| 6 | 10,450 | 23 | 54 | 5,600 | 0.2 | 28 |
| 7 | 17,200 | 12 | 41 | 9,339 | 0.1 | 27 |
| 8 | 8,261 | 15 | 25 | 2,607 | 0.1 | 30 |
| Median | 10,937 | 21 | 39 | 3,741 | 2.1 | 16 |
| Significance | $p<0.0001$ | $p<0.00005$ | $p<0.00005$ | $p<0.001$ | $p<0.005$ | $p<0.005$ |

All results represent the mean of three determinations.

In biological systems, the concentration of redox-active transition metals capable of catalyzing and/or generating free radicals like superoxide, hydrogen peroxide, and hydroxyl radical is relatively low. However, under certain pathological conditions (haemochromatosis, Wilson disease, collagenoses, and various malignancies), transition metals and their transport proteins may accumulate in different target organs inducing cellular lipid peroxidation and DNA-attack. In this respect, the ability of excess Fe in mediating the formation of hydroxyl radicals, suppressing cellular immune functions, and promoting tumor growth is well-established [9, 12, 16, 25]. Increased Cu concentrations were also found in human lung cancer biopsies [1] and in other tumors [5].

Nickel, Cr, and Cd have been recognized as mutagens and carcinogens because of their ability to inhibit the repair of damaged DNA. In addition, they can enhance the mutagenicity and carcinogenecity of directly-acting genotoxic agents [4]. At the same time, carcinogenic effects of Ni, directly or in association with organic compounds, have been described in the literature [6, 15]. Recently, increased concentrations of Fe and Ni have been found in the malignant human prostate [26]. Inhaled particulate forms of hexavalent Cr cause lung cancer, and at cellular level, Cr exposure may lead to cell cycle arrest, apoptosis, or neoplastic transformation [20]. Occupational exposure to Cd is associated with lung cancer in humans, and high Cd concentrations have been found in proliferative prostate lesions [23]. Macromolecular compounds (dextrans) substituted with

Hg-containing side chains were reported to promote fibrosarcoma growth in mice [18].

Interestingly, Zn as an essential element was shown to mediate and increase tumor growth, and Zn depletion was shown to suppress tumor growth in mice and rats [11, 13, 22].

None of our patients were occupationally exposed to metals. However, all were exposed to metals through dental restorations such as amalgam, gold bridges or metallic retainers. Another source of metal exposure is cigarette smoke. About half of our patients were smokers and virtually all have been exposed passively to cigarette smoke.

The higher heavy metal concentrations encountered in various tumor cells may be used for therapeutic interventions with metal chelators, ascorbic acid or phenolic compounds as already reported [3, 7, 8, 10]. Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalyzing free radical reactions according to the equations:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^*OH + OH^-$$

$$H_2O_2 + {}^* O_2^- \xrightarrow{Fe^{3+},\ Cu^{2+}} {}^*OH + OH^- + O_2$$

These Fenton- and Haber-Weiss-reactions are strong generators of hydroxyl radicals leading to lipid peroxidation, DNA strand breaks, and apoptosis [3, 12, 16]. Bioactivation of phenolic/quinonic compounds at the tumor site may lead to a significant generation of superoxide and semiquinone radicals with deleterious action for the metal-rich malignant cells [7, 8]. Preventive diagnostic procedures should include, besides current tumor markers, 2/16-OH-estrogen ratio and Phase II detoxification assessment. Since inflammation often precedes the development of cancerogenic lesions, the MELISA® Test [21] might be useful for the determination of metal-induced inflammation in an individual patient.

In conclusion, the presence of transition metals in breast cancer tissue might be closely related to the malignant growth process.

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