Correlation of Concentrations of Certain Trace Elements in Normal and Diseased Human Tissues

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Abstract

Background Over the past decade there has been a growing awareness of, and interest in, the trace element concentration differences between normal and diseased tissues. Significant changes in tissue concentrations of Zinc (Zn) and Copper (Cu) have been previously reported in inflammation and cancer of certain human tissues.

Aim:(1)To correlate between Zn and Cu concentrations and the histological picture of normal and certain inflamed human tissues, namely the gall bladder (GB) the vermiform appendix (VA), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). (2) to detect whether there is a difference in the above-mentioned parameters between VAT and SAT. (3) to obtain recordings for trace element levels in human tissues.

Methods: Diseased GB (10), VA (10), VAT (10) near these organs and SAT (10) were removed by surgery. Tissues from these organs were then processed for histopathology and analyzed for trace elements concentration by Atomic Absorption Flame-Emission Spectrophotometer.

Results: Zn concentration was high in VAT (0.410 µg/g ± 0.181), GB (0.478 µg/g ±0.531) and VA (0.419 µg/g ±0.123) when compared to its level in SAT (0.1329 µg/g ± 0.0129) and the difference was significant (<0.007,<0.056 and <0.000 respectively).

Cu concentration was high in VAT (0.640 µg/g ± 0.150) and GB (0.919 µg/g ± 0.564) when compared to SAT (0.3893 µg/g ± 0.0130) and the difference was significant (<0.005 and <0.011 respectively). Cu concentration in the VA was low (0.2055 µg/g ±0.0654) and significantly different from all the other tissues (VA vs VAT <0.000, VA vs GB <0.002 and VA vs SAT <0.000).

The histology findings were typical of chronic inflammatory reactions in the GB and of acute inflammation in the VA.

Conclusions: The increase in tissue concentrations of Zn in VAT, GB & VA is due to inflammation.

The high Cu level in chronically inflamed GB and neighboring VAT is due to the increased need for this element during inflammation.

Our results, together with findings reported by others, allow us to think of using trace elements, namely Zn and Cu, as tools for diagnosis and treatment in appropriate conditions.

Key words: Tissue trace elements – Zinc – Copper

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Introduction

Knowledge of the distribution of a trace element in soft tissues is important for biological and clinical applications. Data for such distribution in animal and human tissues is increasing and methods for obtaining this knowledge are different [1, 2, 3]

In addition, a number of projects were performed and reference values for trace elements in human tissues and body fluids were established [4]. However, the number of studies and the recorded data, in normal or diseased human tissues, are still limited.

Most efforts were concentrated on the distribution of different trace elements in normal tissues and little research was done to localize or measure the concentration of trace elements in diseased tissue.

Over the past decade there has been a growing awareness of, and interest in, the trace element concentration differences between normal and diseased tissues.

In a number of studies on lung cancer significant differences in trace element content were found between malignant and non malignant lung tissue [5, 6, 7]

In another study, the concentrations of Mn, Fe, Cu, and Zn were correlated with the clinical stage of prostate cancer and the feasibility of their use as a potential diagnostic marker in the different stages of prostate diseases was discussed [8].

In other studies on lung diseases Serum zinc was suggested as a marker of lung disease [9,

[10], and sputum zinc as a biomarker of suppurative inflammatory diseases, as cystic fibrosis [11].

Our aim in this study was to correlate between the concentrations of certain trace elements, namely Zn and Cu, and the histological picture of inflamed gall bladder (GB) vermiform appendix (VA), visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in human. In addition we aimed to detect whether there was a difference in the above-mentioned parameters between VAT and SAT. and to obtain recordings for trace element levels in human tissues.

Methods:

The GB and VA were removed by surgery from patients diagnosed of having cholecystitis (10 patients) and appendicitis (10 patients), of different age and sex, , in Al Kindy Teaching Hospital in Baghdad. VAT was removed together with the diseased organ, together with a piece of normal SAT. Tissues were then processed for histopathology and analyzed for trace elements concentration by Atomic Absorption Flame-Emission Spectrophotometer.

Histopathology

The tissue was cut into small pieces (0.5 cm3), fixed in 10% formalin and embedded in paraffin. The paraffin sections were cut into 4 µm thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed using Leica (CM E) Compound light Microscope with an attached digital camera (DCM310).

Trace elements Analysis

Atomic Absorption Flame -Emission Spectrophotometer model AA-6200 Shimadzu-Japan was used for measurement of the concentration of Zn and Cu in GB, VA, VAT and SAT. 100ul of wet ash sample was diluted with de-ionized water 100 times and analyzed for trace elements concentrations (µg/g).

Statistical Analysis

Data was entered into computer and analyzed by Minitab version 13.

The means and standard deviation were calculated and the T-test was used to analyze the difference between the means of the groups. P-values less than 0.05 was considered statistically significant.

Results:

Zn concentration:

Zn concentration in VAT was (Mean: 0.410 µg/g ± SD: 0.181), in GB it was (Mean: 0.478 µg/g ± SD: 0.531) and in VA it was (Mean: 0.419 µg/g ± SD: 0.123). The difference in Zn concentrations between the different tissues was not significant.

Zn concentration in SAT was (Mean: 0.1329 µg/g ± SD: 0.0129) a level that was significantly lower when compared to its level in VAT, GB and VA ($P<0.007$, $P<0.056$ and $P<0.000$ respectively). (Table 1)

Cu concentration:

Cu concentration in VAT was (Mean: 0.640 µg/g ± SD: 0.150) and in GB it was (Mean: 0.919 µg/g ± SD: 0.564), a non significant difference between the two levels.

Cu concentration in SAT was (Mean: 0.3893 µg/g ± SD: 0.0130), a level that was low and of significant difference when compared to VAT and GB ($P<0.005$ and $P<0.011$ respectively).

Cu concentration in the VA was (Mean: 0.2055 µg/g ± SD: 0.0654) a level that was significantly lower than its level in all the other tissues (VA vs VAT $P<0.000$, VA vs GB $P<0.002$ and VA vs SAT $P<0.000$). (Table 2)

Histology:

In the GB, the histology findings were of typical chronic inflammation. The blood vessels were dilated and the tissues were heavily infiltrated with lymphocytes and macrophages Figure 1.

In the VA, the histology picture was that of acute inflammatory reaction with dilated blood vessels, polymorpho-nuclear cell infiltration and cell debres Figure 2.

Histology of VAT and SAT was that of normal adipose cells and non congested blood vessels. No inflammatory cells infiltration was seen.

Discussion

High concentrations of Zn in tissues present in the area of inflammation are caused by the inflammatory process. Inflammation increases Zn uptake to the involved area and the nearby tissue. This is because Zn has anti-inflammatory effects proved by many studies and explained to be through different mechanisms [12, 13].Zn diversely acts as an antioxidant [14, 15], an anti-apopototic agent [16] and an anti-inflammatory agent [17, 18, 19, 20].

In one study linking asthma with (Zn) it was reported that inflammation-induced alterations in Zn transporter gene expression are directed toward increasing Zn uptake. Increases in Zn uptake may be needed to counteract the local loss of Zn in the airway and to meet an increased demand for Zn-dependent proteins [19], and that in contrast to many anti-inflammatory drugs, zinc does not suppress, but improves immune reaction upon pathogen invasion. These results suggested that mildly zinc-deficient, healthy elderly subjects might benefit from moderate zinc supplementation due to a more balanced immune response with reduced incidences of infections and autoimmune diseases [20, 21]. Also, other studies suggested that zinc nutrition can markedly modulate mechanisms of the pathology of inflammatory diseases such as atherosclerosis[22].

The mechanisms by which Zn produces its effects are numerous and can be reviewed here. Zinc is essential for the structure and function of literally hundreds of proteins [23]. Therefore, changes in zinc availability alter numerous cellular processes. Multiple genes have evolved to control the storage (Metallothionein genes), efflux (Slc30a; Znt genes) and uptake (Slc39a; Zip genes) of this metal ([24]).

Recent studies also reveal that zinc can be stored and released from intracellular vesicular compartments and can function as a novel intracellular second messenger [25, 26]. Zinc can modulate the proliferation and differentiation of mammalian cells by affecting several growth factor signaling cascades [27].

Our results show that Zn concentrations in the inflamed GB, VA and the VAT taken from neighboring areas to inflammation, are similar and non-significantly different. These values are significantly greater than the element concentrations in SAT, which is taken from the same patient but from a distant region not involved in the area of inflammation, and thus can be considered as the normal tissue.

The higher values of Cu concentrations in GB and VAT found in our results are caused by the inflammatory process is due to the increased need for this element during inflammation.

Copper (Cu) is an essential trace element that can cycle between reduced (Cu+) and oxidized (Cu2+) forms. This property allows Cu to act as a catalytic co-factor for several metallo-enzymes involved in a number of biochemical processes including cellular respiration, connective tissue formation, neurotransmitter production, pigment synthesis, antioxidant defense and iron homeostasis [28].

Acute and chronic inflammation are characterized by changes in the metabolism of Cu and by a pronounced responsiveness to therapy with copper compounds [29, 30].

Cu content and ceruloplasmin activity of serum are significantly elevated above normal values in inflammatory diseases in humans and laboratory animals, and Cu in widely different chemical forms is used as

therapeutic agent in therapy for chronic and acute inflammation [30].

In one study, there was a rise in total serum Cu in inflammation and it was suggested to represent the natural anti-inflammatory response of the organism itself. The responsiveness of inflammatory disorders to Cu supplementation suggested that the control exerted by endogenous Cu on inflammation was susceptible to enhancement by exogenous sources [31, 32].

However, a low Cu content in the inflamed appendix may be due to different mechanisms in Cu metabolism in different tissues, a matter that needs further research.

From our results we can conclude that trace elements, namely Zn and Cu, are differently distributed between normal and inflamed tissues. The higher levels of concentration are found in the inflamed tissues because of a higher tissue uptake of the elements. This higher uptake is part of the body defense mechanism against the pathogenic insult due to the anti-inflammatory actions of these elements.

These findings allow us to think of using these trace elements as tools for diagnosis.

In addition, these findings demonstrate the necessity for more thorough and complete screenings for trace elements in other normal and diseased human tissues.

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