Influence of some Trace Elements and Biochemical Parameters on Breast Cancer

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الخلاصة

تهتم الدراسة بتقدير مستوى المعادن ال ن ادرة، إضافة الى مستوى البروتين الكلي والكوليستيرول في مصل الدم التي قد تعتبر مؤشرات كيموحيوية للإصابة بسرطان الثدي. حيث جمعت النماذج لمصل الدم من ٢٥ امرأة من النساء السليمات و ٢٥ امرأة من النساء المصابات بسرطان الثدي، قبل ٢٤ ساعة من إجراء العملية الجراحية لإستئصال الثدي . وتم تقدير مستوى كل من العناصر الن ادرة التالية: Cu و Zn و Pb و Fe بتطبيق تقنية الامتصاص الذري . كما تم تقدير مستوى كل من البروتين الكلي والكوليستيرول الكلي وكوليستيرول البروتين الدهني عالي الكثافة في مصل الدم بالطريقة الانزيمية . مقارنة بالنساء السليمات، أظهرت النساء المصابات بسرطان الثدي ارتفاعا معنويا في مستوى Cu ($P < 0.009$) و Pb ($P < 0.03$) والنسبة Cu/Zn ($P < 0.001$) ومستوى البروتين الكلي ($P < 0.005$) والكوليستيرول الكلي ($P < 0.001$)ونسبة الكوليستيرول الكلي/كوليستيرول البروتين الدهني عالي الكثافة ($P < 0.05$). كما أظهرت النساء المصابات انخفاضا معنويا في مستوى Zn ($P < 0.002$) لكنها لم تظهر تغييرا معنويا في مستوى Fe و نسبة Cu/Fe ومستوى كوليستيرول البروتين الدهني عالي الكثافة . إستنادا الى هذه النتائج يمكن ا لاستنتاج بأن نوع ومستوى العناصر ال ن ادرة، إضافة الى مستوى البروتين والكوليستيرول قد يكون لها علاقة بلحتمالية الإصابة بسرطان الثدي، ح يث ان المستوى العالي لكل من Cu و Pb ونسبة Cu/Zn ومستوى البروتين الكلي والكوليستيرول الكلي ونسبة الكوليستيرول الكلي /كوليستيرول البروتين الدهني عالي الكثافة، إضافة الى المستوى المنخفض للعنصر Zn قد يكون لها علاقة بزيادة احتمالية الإصابة بسرطان الثدي.

Abstract

The study investigates the levels of serum trace elements as well as total protein and cholesterol which may be regarded as biochemical markers in the case of breast cancer. Serum samples were collected from 25 healthy females and 25 females with breast cancer, 24 hours before mastectomy surgery. Serum Cu, Zn, Pb and Fe were measured by using the atomic absorption spectrophotometer. Total protein, total cholesterol and HDL-cholesterol were analyzed enzymatically. Compared with the healthy females, the females with breast cancer exhibited a significant increase in the level of Cu ($p < 0.009$), Pb ($p < 0.03$), Cu/Zn ratio ($p < 0.001$), total protein ($p < 0.005$), total cholesterol ($p < 0.001$) and total cholesterol/HDL-cholesterol ratio ($p < 0.05$). They also exhibited a significant decrease in the level of Zn ($p < 0.002$), but there was no significant change in the level of Fe, Cu/Fe ratio and HDL-cholesterol. It was concluded that the type and level of the elements and the level of protein and cholesterol might have a correlation with breast cancer; a high level of Cu, Pb, Cu/Zn ratio, total protein, total cholesterol and total cholesterol/HDL-cholesterol ratio, and a low level of Zn may be associated with an increased risk of breast cancer.

Introduction

At least one-third of all human cancer may be associated with diet and influenced by lifestyle and physical exercise [1].

One of the most common malignancies in women is breast cancer which is considered to be the leading cancer-related cause of death among women in most developed countries [2].

Unfortunately, there has been no effective therapy to prevent this disease. Given this, a large number of epidemiological studies have been undertaken to identify the potential risk factor for cancer [3].

Although there has been a growing recognition that metal compounds are an important class of environmental and occupational carcinogens [4], trace elements or heavy metals have received little attention [2,5]. Trace elements are present in minute quantities, but they play a vital role in many biochemical enzymatic reactions and have been examined critically as a potential key factor in various human diseases including cancer [6]. Trace elements may contribute to tissue carcinogenesis due to their role in regulating cell proliferation, differentiation and apoptosis [7]. Trace elements, such as copper, zinc, lead and iron, are found naturally in the environment, and human exposure derives from a variety of sources including air, drinking water and food [5].

On the other hand, numerous studies implicated a role for cholesterol in the mechanism underlying cell proliferation and cancer progression [8,9,10]. In addition, there is an inverse change in the level of serum-protein fractions as a result of the acute phase response in which levels of

several proteins increase or decrease in response to the physiological stresses, such as tissue necrosis and inflammatory conditions [11, 12, 13].

The present study focuses on the correlation between the level of the trace elements noted above, in addition to the level of cholesterol and proteins, of Iraqi females and the probability of causing breast cancer.

Materials and Methods

Subjects

The study was conducted on free living subjects and was not strictly controlled for nutrients and energy intake. The subjects included two groups, patient and healthy, of Iraqi females aged 40-60 y. The patient group consisted of 25 females with diagnosed breast cancer (irrespective of its stage), 24 hours before mastectomy surgery, and they were visitors to the surgery clinics of the Al-Zahrawi Teaching Hospital in Mosul province. The healthy (control) group consisted of 25 apparently healthy females which were recruited mostly from members of staff of the hospital. The study excluded patient or controls with any drug therapy.

Blood Sampling and Analysis

Venous blood samples were drawn at the approval of the hospital management, from the females who showed no objection, into plain tubes, and the serum was separated 2h after venipuncture by centrifugation at 3500 rpm for 5 min, then it was stored at -20^{o}C until ready for assay.

The levels of serum trace elements (viz, Cu, Zn, Pb and Fe) were determined using the atomic absorption spectrophotometer [14].

The levels of serum total cholesterol (TC), HDL-Cholesterol (HDL-C) and total protein were analyzed enzymatically using commercial reagents (kits obtained from BioMerieux, France).

Statistical Analysis

The data was subjected to statistical analysis using the student unpaired t-test for comparison of means between patients and controls. All the data were expressed as mean ± standard deviation of the mean. P-values ≤ 0.05 were considered significant.

Results

The level of serum trace elements (viz. Cu, Zn, Pb and Fe), the Cu/Zn ratio and the Cu/Fe ratio of healthy females (control group) and females with breast cancer (patient group) are shown in Table1. Compared with the healthy females, the females with breast cancer exhibited a significant increase in the level of Cu ($P<0.009$), Pb ($P<0.03$) and the Cu/Zn ratio ($P<0.001$). They also exhibited a significant decrease in the level of Zn

(P<0.002), but there was no significant change in the level of Fe and the Cu/Fe ratio.

Table (1): Serum-trace elements levels

Serum trace elements	Mean ± SD		*P-value
	Healthy women (n 25)	Breast cancer women (n 25)	
Cu μg/100ml	119.344 ± 19.640	173.928 ± 32.376 ↑	0.0086
Zn μg/100ml	115.872 ± 19.296	81.384 ± 19.765 ↓	0.0019
Pb μg/100ml	0.624 ± 0.347	1.056 ± 0.586 ↑	0.028
Fe μg/100ml	165.234 ± 21.005	177.808 ± 16.632	0.246
Cu/Zn ratio	1.029 ± 0.4011	2.129 ± 0.765 ↑	0.0009
Cu/Fe ratio	0.782 ± 0.378	0.928 ± 0.401	0.066

*P ≤ 0.05 considered significant.

Table 2 shows the levels of the analyzed serum biochemical parameters (viz. TC, HDL-C, total protein and the TC/HDL-C ratio). Compared with the healthy females, the females with breast cancer exhibited a significant increase in the level of total protein and TC (P<0.001) and the TC/HDL-C ratio (P<0.05). They also exhibited no significant change in the level of HDL-C.

Table (2): Some Serum Clinical Characteristics

Serum Clinical Characteristics	Mean ± SD		*P-value
	Healthy women (n 25)	Breast cancer women (n 25)	
Total protein gm/dl	6.655 ± 0.362	7.791 ± 1.489	0.004
Total cholesterol mmol/L	4.468 ± 0.480	5.936 ± 1.098	0.001
HDL-cholesterol mmol/L	0.998 ± 0.222	1.106 ± 0.292	0.146
Total cholesterol / HDL-cholesterol	4.721 ± 1.270	5.686 ± 1.699	0.027

*P ≤ 0.05 considered significant

Discussion

The rate at which breast cancer is spreading like an epidemic is alarming and there is a large number of cases reported in recent years [2]. This observation calls for urgent control and management of this type of cancer.

A decline in the cell-mediated immunity predisposes to oncogenesis [15], and a close association has been found between immune responses and macro- or micronutrient status [16].

This implies that it may be possible to monitor the prognosis of cancers using the levels of trace elements [1]. The present study is limited to just serum Cu, Zn, Pb and Fe trace elements, in addition to serum protein and cholesterol.

The findings shown in Table 1 indicate a strong association of Cu with breast cancer. Women with breast cancer exhibited a significant increase in the level of Cu ($P<0.009$) as compared to the controls. The elevation of Cu level in breast carcinoma has been reported by numerous investigators [1, 3, 4, 17]. It has been suggested that the copper ions and copper complexes react with hydrogen peroxide to form hydroxyl radicals that cause damage to protein, RNA and DNA. The damages are not repairable by cellular mechanisms thus initiating the malignant process [18]. In addition, angiogenesis (the growth of tumor blood supply) is essential for tumor growth, invasion and metastasis [19-21]. Molecular processes of angiogenesis that require copper as an essential cofactor for the stimulation of endothelial growth by tumor cytokine production (i.e., vasoendothelial growth factor), degradation of extracellular matrix proteins by metalloproteinases and migration of endothelial cells are mediated by integrins [22-24]. Consistently, high levels of copper have been found in many types of human cancers, including breast, prostate, colon and brain [25-27]. In human tumor-cell culture, it has been found that organic copper compounds inhibited the proteasome activity very rapidly (15 min) followed by induction of apoptosis [28]. So, we can say that although copper is an essential trace element for animals, its amount in an organism must be tightly regulated [29].

The serum-Zn level was significantly lower in the breast-cancer group ($p<0.002$) than in the control as shown in Table 1. This finding agrees with that reported by some investigators [4, 17, 30,31] but not with others [1, 3, 32]. The exact role of Zn in carcinogenesis is unknown [4]. However, Zn is known to be essential for more than a hundred different metabolic functions [3, 33, 34]. It is required for DNA synthesis by altering the binding of F and F_3 histones to DNA so as to affect RNA synthesis [35]. It is also essential for the activation of adenyl kinase, phosphodiesterase, membrane-bound adenyl cyclase and lipid peroxidase [3, 4]. Experimentally, Zn deficiency and Zn supplementation have each shown both inhibition and stimulatory responses on tumor growth [3, 35], adding confusion to the role of Zn in human cancer.

The level of Pb was significantly elevated in the breast-cancer group ($p<0.03$) compared to the healthy group. During the past few decades, there has been growing recognition that metal compounds are an important class of environmental and occupational carcinogens [36, 37], and lead has been used to induce cancers in experimental animals [38]. Many of these studies have indicated that metal ions interact with nucleic

acids to influence base-pairing and conformation. Such effects have been known to cause somatic mutation, a consequence of base-pairing errors of frame-shift mutations by deletion, leading to cellular transformation [39].

Table 1 shows that the level of Fe was insignificantly higher in the breast-cancer group. This finding agrees with that reported in previous studies [1, 3, 32]. This makes iron, as Becker *et al* [5] also suggest, a weak biomarker in the case of breast cancer.

The increased awareness of the role of trace elements and their interactions in metabolism and disease need a better understanding of the interrelationships of these metals for better understanding of their role in regulating tumor growth [3]. For trying to achieve this goal, we determined the Cu/Zn ratio and the Cu/Fe ratio. The latter ratio reflected insignificant change while the Cu/Zn ratio exhibited a higher increase in the breast-cancer group. This finding is in agreement with that found by Lonesco and *et al* [37].

Table 2 shows that there were marked differences in the levels of total protein and total cholesterol (TC), and in the TC/HDL-C ratio for the healthy group and the breast-cancer group. The latter group exhibited a significantly higher level of total protein ($p<0.005$). Clinical hematological abnormalities have been reported in breast-cancer cases [40], which may lead to the elevation of proteins. This elevation agrees with that reported by Laursen *et al* [41], and it was stated that many globulin fractions were high in cancer cases [42]. Watabe [43] reported that the average level of α_1-globulin in rat-serum during carcinogenesis was 2-4 mg/dl at the 6^{th} week, but after 13 weeks of carcinogenesis, it reached 60-100 mg/dl.

Concerning serum TC, Table 2 shows that its level was significantly higher ($p<0.001$) in the breast-cancer group. This finding agrees with that reported by Hardwick *et al* [44] who state that cholesterol plays an important role in the mechanism underlying cell proliferation and cancer progression. It has been stated that high serum cholesterol, particularly in combination with properative weight, is a significant prognostic determinant of breast cancer [45].

Table 2 exhibits an insignificant increase in HDL-C, but Rossner and Wallgren [46] found that breast-cancer patients had a significantly higher level of serum cholesterol than the controls, 16% higher level in LDL-C and 13% higher level in HDL-C. On the other hand, it was stated that low HDL-C is associated with increased postmenopausal breast-cancer risk [47]. There are abundant data showing that animals fed fat diets rich in saturated, trans- or n6-fatty acids show a decrease in HDL-C and develop lifestyle-related cancers (breast, intestine, pancreas and colorectal) more readily than animals fed fat diets poor in these fatty acids [48]. The confusion in these results may be attributed to the wide age-range of the

individuals used in the present study (including menarche and menapouse women), which has a relation to the level of TC, LDL-C and HDL-C [49]. To minimize the confusion, the ratio of TC/HDL-C was taken to consideration. The results shown in Table 2 exhibit a significant increase ($p<0.005$) in the amount of the ratio in the breast-cancer group compared to the control group, which reflects a positive correlation with breast-cancer incidence.

In conclusion, the type and the level of some trace elements and the level of serum protein and cholesterol might have a correlation with breast cancer; the high level of Cu, Pb, Cu/Zn ratio, total protein, total cholesterol and TC/HDL-C ratio, and the low level of Zn may be associated with an increased risk of breast cancer.

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