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QUANTITATIVE ANALYSIS OF NUCLEOSIDES AND TRACE ELEMENTS IN SALIVA OF BREAST CANCER PATIENTS

***Abdul-Wahab R. Hamad**

College of Pharmacy, Muta'h University, Al-Karak, Jordan.

***Corresponding: Abdul-Wahab R. Hamad**

College of Pharmacy, Muta'h University, Al-Karak, Jordan.

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ABSTRACT

Background: Several attempts made to establish methods of diagnosis for different diseases by the compositional analysis of saliva, which investigated as a supplementary to serum for diagnostic and epidemiological testing of different diseases. Most molecules found in blood and urine are found in saliva, but their concentrations are estimated to be one tenth to one thousandth that of blood. **Materials and Methods:** Determination of Nucleosides [(Pseudouridine (Ψ), Cytidine (C), 3methylcytidine (m3C), 1-methyladenosine (m1A), 2-thiocytidine (m5C), 5-methylquanosine (m7G), Inosine (I), Rhibothymidine (T), 1-methyllinosine (mII), 1-methylquanosine (m2/2G)] by using HPLC. Measured some essential trace elements (copper, zinc, Iron, chromium, manganese, magnesium, cobalt and selenium) levels by using Atomic Absorption. Both measurements have done in 50 breast cancer (BRCA) female patients and compared with 50 healthy women as controls. Saliva samples from two groups (control and patients) were prepared and quantitative determination of Nucleosides and Trace Elements. Nucleosides were determined by using High Performance Liquid Chromatography (HPLC), while the trace elements, copper, zinc, Iron, chromium, manganese, magnesium, cobalt and selenium determined by Flame and Flameless Absorption Atomic Spectrophotometer. **Results:** It is found that nucleosides (especially, Pseudouridine, 1-Methyl Inosine, 1-MethylGuanosine, N-N-Dimethyl- Guanosine), a significantly increased ($p < 0.05$) in patients compared with normal subjects. Copper concentration was higher in breast cancer patients, while iron, zinc, magnesium, manganese, selenium, chromium, and cobalt levels have shown a remarkable decrease in those patients when compared to the control group, and the metal-to-metal ratio measured in this work is very indicative in studied. Saliva Cu / Zn ratio was significantly increased compared to healthy control subjects. **Conclusion:** from this study, the suggestion is a saliva pseudouridine and some trace elements might be a useful as specific markers for breast cancer disease. This method can used for monitoring patients with malignant disease and for monitoring clinical response to chemotherapy and radiotherapy or both.

KEYWORDS: Saliva samples from two groups (control and patients) were prepared and quantitative determination of Nucleosides and Trace Elements.

INTRODUCTION

Cancer is the uncontrolled growth of cells that produces a tumor or neoplasm.[1,2] Cancer cells lose their tissue specialization and do not respond to the control mechanisms that normally limit cell division. In addition, cancer can spread from the primary tumor and develop into secondary tumors in a process called metastasis.[2,3,4]

Breast cancer happens when cells in breast tissues begin to grow out of control and can then invade nearby tissues or spread throughout the body. Large collections of this out of control tissue that called tumors.[3,5,6]

Breast cancer is the first leading type of cancer in women followed by lung cancer as shown in Table 1 below.

Cancer cells can invade nearby tissues and spread (metastasize) through the bloodstream or lymphatic system to distant organs like the lungs, liver, or bones. Factors increasing breast cancer risk include age, family history, specific genetic mutations, and certain lifestyle choices like a high-fat diet or heavy alcohol use.[7,8]

Signaling factors have been extensively studied in recent years to investigate their role in cancer etiology. Magnesium and zinc are crucial factors in regulating cell growth, division, and differentiation. High levels of copper have been shown to protect against the chemical induction of tumors.[9] The correlation between serum copper and disease grade has indicated that serum copper could act as a tumor marker or tumor antigen, according to a study by Thorling EB and Thorling K.[10]

Trace elements have been studied in recent years to evaluate their role in cancer etiology. Magnesium and zinc are essential elements in the regulation of cell growth, division, and differentiation. High levels of copper have observed to protect against chemically induced tumors.[9] Contrary to claims made by some users, scientific studies suggest that high levels of copper in tissues and serum are generally associated with cancer progression, tumor growth, and metastasis, rather than providing protection against chemically induced tumors. Excessive copper levels may promote tumor growth by meeting metabolic demands, favoring angiogenesis, disrupting tumor suppressor proteins, and correlate with more advanced stages of cancer.[10,11,12]

The trace elements have extensively researched in recent years to determine if they play any part in the development of cancer. Magnesium and zinc are the elements that significantly influence the processes of cell growth, division, and differentiation. Elevated levels of copper have observed to provide protection against chemically induced tumors.[13]

The relationship between serum copper levels and disease progression has suggested that serum copper could act as a tumor marker or a tumor antigen, according to research carried out by Thorling EB and Thorling K.[10]

The association between serum copper and the advancement of the condition has pointed to the possibility that serum copper may be utilized as a tumor marker or a tumor antigen, in research conducted by Thorling EB and Thorling K.[10]

Khanna and Karjodkar,[14] in their research, determined that there was a rise in the concentrations of circulating immune complexes in individuals with precancerous conditions and cancer, that the serum copper levels progressively increased from precancerous to cancer patients, and that the serum iron levels were notably lower in those with cancer. 15. Fisher GL et al.[15] concluded that the correlation between serum copper and serum zinc levels could be beneficial in distinguishing between patients suffering from primary and metastatic osteosarcomas.

Khanna S and Karjodkar F,[14] in their research, found that there was an increase in the levels of CICs among individuals with precancer and cancer, that there was a steady rise in serum copper levels from precancer to cancer patients, and that serum iron levels were notably lower in those with cancer. Fisher GL et al. 1976[15] concluded that the relationship between serum copper levels and serum zinc levels could be beneficial in distinguishing between patients with primary and metastatic osteosarcomas.

Vyas RK et al.,[16] concluded that the zinc, serum magnesium and the calcium levels found to be low in the patients with malignancies and cirrhosis and concluded that the zinc, serum magnesium and the calcium degrees were determined to be low inside the sufferers with malignancies and cirrhosis.

Oyama T, Kawamoto T. et al.,[17] in their study, concluded that a preoperative increase in the Cu/Zn levels predicted the tumour progression better than the changes in the Cu or the Zn levels. The measurement of Cu/Zn was useful for assessing both the extent and the prognosis of the disease in NSCLC patients, which was similar to the measurement of the tumour markers such as CEA. The measurement of Cu/Zn had a prognostic significance.[18]

Rajendran R, Babu KN, Nair KM (19) discovered excessive stages of magnesium, cadmium and selenium in Oral submucous fibrosis OSMF. They said that elevated stages of cadmium and selenium in OSMF is probably part of a mechanism, which turned into worried within side the malignant transformation of OSMF.

Oyama T, Kawamoto T et al.,[17] in their study, concluded that a preoperative growth inside the Cu/Zn stages expected the tumour progression higher than the changes inside the Cu or the Zn degrees. The measurement of Cu/Zn turned into useful for assessing each the volume and the prognosis of the disease in NSCLC (Non-small cell lung cancer) sufferers, which become much like the measurement of the tumour markers consisting of CEA.

Table 1: the incidence of the five major cancer cases in women in North America and Europe.[4]

Cancer	Percentage %	Risk Factors	Possible Mechanisms
Breast	33	Sex hormones, Family history Diet	Proliferation Gene defects Hormones
Lung	13	Smoking	DNA damage
Colon	12	Family history	Gene defects, Antioxidants
Leukemia	6		Free radical, chemical induce cancer
Ovary	5	Ovulation Family history	Tissue damage, Gene defects

MATERIALS AND METHODS

Subjects

One hundred individuals employed in this study; they included fifty women patients diagnosed clinically and histologically as breast cancer, their age range between 24 – 65 years. Fifty age and sex matched normal individuals were used as control group.

Unstimulated (resting) whole saliva collected after the patients have rinsed their mouth several times with tape water. The collected samples centrifuged at 2500 rpm for 10 minutes within one-hour collection and the centrifugal supernatant deep-frozen at -20°C until time of analysis.

The instrument used in this work was

a. Flame and Flameless Atomic absorption spectrophotometer (Shimadzu, Japan) for determination of Zn, Cu, Fe, Mn, Co, Cr and Mg. Model 6800 fitted with nitrous oxide – acetylene burner head. Hollow cathode lamps used as radiation emission sources for Zn, Cu, Fe, Mn, Co, Cr and Mg. Absorption measured in a Fuel- rich flame in order to obtain maximum sensitivity.

This instrument (as flameless) was equipped with a heated graphite furnace and used for determination of Selenium. Highly purified argon gas used as purge gas at rate of flow of 50 Ml/min.

b. The Chromatographic System consisted of LC-10A high performance of chromatography SPD 10A UV-Vis spectrometric detector and data processor (all obtained from Shimadzu Co., Kyoto, Japan). The column was C-18; particles size was 5 um (Waters Assoc., Milford, MA).

The mobile phase was 0.03M ammonium phosphate with 2.5% methanol adjusted to pH 5.1, flow rate 1 ml/min. and the absorbency intensity monitored at 254 nm. All buffers filtered through milliporefilter 0.22um and degassed by purging the mobile phase for 5 minutes with Helium gas before used. The separation temperature was at 42°C.

Pseudouridine (Ψ), Cytidine (C), 3methylcytidine (m3C), 1-methyladenosine (m1A), 2-thiocytidine (m5C), 5-methylquanosine (m7G), Inosine (I), Rhibothymidine (T), 1-methyllinosine (mII), 1-methylquanosine (m2/2G) were purchased from Suplec Co., USA.

RESULTS AND DISCUSSION

a. Nucleosides

Elevations in the concentrations of specific nucleosides (Table 1), notably pseudouridine, 1-methylinosine, 1-methylguanosine, and N-N-dimethylguanosine, found in the saliva of individuals with breast cancer were statistically very important, aligning with increased levels observed in the serum of patients suffering from different cancer forms.[20,21] in the urine of individuals suffering from different forms of cancer,[22] and in the fluid present in malignant pleural effusions.[23] The influence of clinical status on pseudouridine can certainly be used as an indicator of early detection of malignant transformation and as a means of determining tumor response to therapy. Furthermore, it may be a valuable marker for monitoring the course of the disease during treatment. The increased level of modified nucleosides (1-methylinosine, 1-methylguanosine and N-N dimethylguanosine) in saliva samples of breast cancer patients may also serve as a diagnostic marker in the detection of breast cancer.[24]

b. Trace Elements

Data obtained from the saliva of women with breast cancer compared with controls (Table 2). Salivary levels of Zn, Fe, Se, Mg, Cr, Mn, and Co were significantly lower ($p > 0.05$) in breast cancer patients. However, Cu levels were significantly higher in the patient group compared with healthy women (Table 2). Many enzymes attracted to the cell membrane, controlling its function and structure, however, zinc controls the activity of these enzymes (26). Therefore, Zn deficiency causes membrane damage and is important as an antioxidant in the enzyme (Cu-Zn SOD) (copper-zinc superoxide dismutase) to protect cellular structure from oxidative damage.

Zinc deficiency affects DNA damage, oxidative stress, antioxidant defense mechanisms, and DNA repair in rats[27] 15a Yang Song 4, Scott W Leonard 5, Maret G Traber 4,5, Emily Ho. J Nutr., 2009; 139(9): 1626–1631.

The association between cancer development and low serum zinc concentrations can be explained[28]

It is possible to explain the relation between the development of cancer and low concentration of serum zinc. Copper in plasma or serum occurs in two main forms: one bound to serum albumin, the concentration of which increases after Cu ingestion, and the largest amount of serum Cu is permanently bound (80–95%) to globulin. This globulin, called ceruloplasmin, is a metalloprotein whose concentration remains constant even in the presence of dietary copper deficiency because albumin can readily release copper into tissues.[29] Copper plays a role in angiogenesis, which is essential for new tissue development and tumor growth.[30] Cu ions have implicated in biological damage caused by superoxide, a radical present in all living tissues.[31]

Iron deficiency causes anemia, which occurs with reduced transport and a generalized decrease in plasma protein levels due to various causes. These factors may include reduced protein intake and reduced protein synthesis resulting from zinc deficiency, which stimulates protein catabolism. Selenium reduces the incidence of cancerous tumors in animals exposed to various chemical carcinogens.[32] Another study found that selenium inhibits both the initiation and promotion phases of carcinogenesis, and continuous selenium supplementation is necessary to achieve maximum inhibition of tumor development.[33] As a component of selenium-dependent antioxidant enzymes, selenium helps neutralize free radicals, thus preventing cell damage. One theory of cancer is that damage to cellular DNA by free radicals may lead to the development of certain cancers. Hanna and Harrison were aware of the significant protective effects of selenium supplementation and other antioxidants.[34] Cobalt is essential for human as an integral part of vitamin B12. The decrease in Co levels may be due to its carcinogenic functions.

Several studies have found that disturbances in magnesium metabolism significantly affect the central and peripheral nervous systems in humans.[33]. Reduced magnesium levels may be explained by the increased metabolic demand for magnesium in cancer cells and tissues, as magnesium is an essential cofactor for 300 enzymes. This increase may explained by the importance of magnesium for the specific area (tumor) and its role in cancer tissue growth.[34] Mg is an essential factor for DNA, RNA, and ribosomes, and is required for the binding of RNA to polysomes during protein synthesis.[35] Manganese considered an anti-cancer factor for several enzymes, such as Mn-SOD,[36] the main mitochondrial antioxidant enzyme, which plays a role in protecting mitochondria from oxidative damage (as mitochondria consume over 90% of the oxygen used by cells).

Trace elements and nucleosides mainly engage at the point of nucleotide metabolism and the capability of enzymes, whereby trace elements serve as vital cofactors for enzymes that play a role in the creation and control of nucleotides, which are derived from nucleosides alongside phosphate.[35]

The Binding of Copper (II) to Nucleosides, Nucleotides, and Deoxyribonucleic acids of Metal Ions with Polynucleotides and Related Compounds. VII. The Binding of Copper (II) to Nucleosides.[36]

Our conclusion, that quantitative analysis of various nucleosides and trace elements found in saliva can used as tumor markers not only for breast cancer but for other malignancies as well.

Table 1: Mean concentration and standard deviation of Nucleosides in Saliva of breast cancer patients and normal.

Pvalue	Patients- Mean±SD (nmole/ml)	Healthy- Mean±SD (nmole/ml)	No., of samples	Nucleosides
0.01<	0.605± 0.130	0.210± 0.047	50	Pseudouridine
0.01<	0.71± 0.020	0.003± 0.0005	50	1-Methyl Inosine
0.01<	0.069± 0.030	0.006± 0.0017	50	1-MethylGuanosine
0.01<	0.063± 0.024	0.003± 0.0005	50	N-N-Dimethyl- Guanosine

Table 2: Mean concentration of Trace elements in Saliva of Breast Cancer and Healthy Women.

Pvalue	Patients Mean ± SD ug/ml	Healthy Mean ± SD ug/ml	No., of samples	Trace elements
<0.01	0.19±0.04	0.31±0.05	50	Zn
<0.01	0.23±0.1	0.13±0.09	50	Cu
>0.05	0.14±0.03	0.31 ±0.07	50	Fe
>0.05	0.019±0.002	0.074±0.002	50	Se
<0.01	4.605±1.77	6.579±0.776	50	Mg
<0.05	0.020±0.01	0.050±0.01	50	Cr
<0.05	0.007±0.002	0.15±0.03	50	Mn
<0.05	0.021±0.029	0.034±0.001	50	Co
<0.01	0.60 ± 0.50	1.25±0.63	50	Cu / Zn

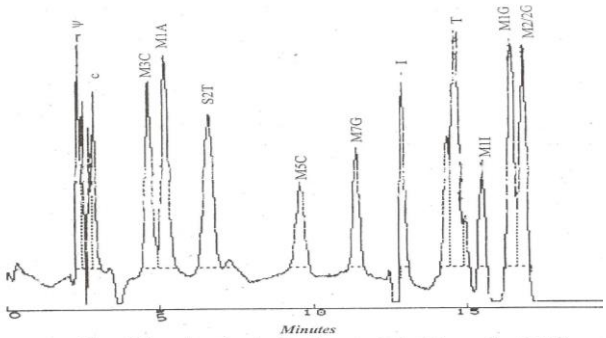

Figure 1 Separation of nucleosides from standard solution on C – 18 DBS column (250 X 4.6 mm I.D.) mobile phase; 0.03 M ammonium phosphate with 2.5%, flow rate 1 ml/min. absorbancy at 254 nm, temp. 42 °C.

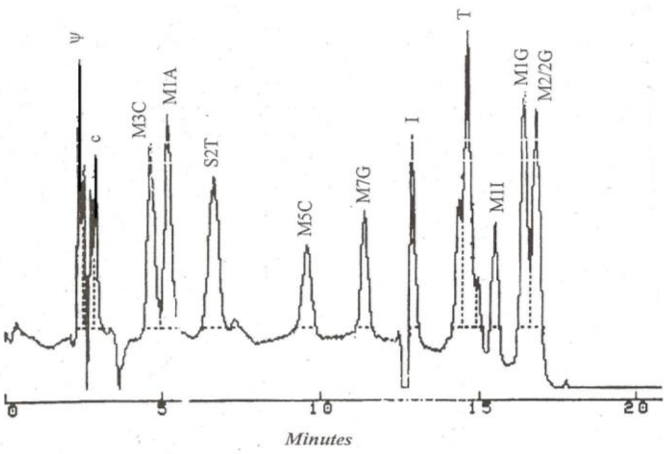

Figure 2 Chromatogram of nucleosides' separation from saliva of normal healthy women. Chromatographic conditions are the same as in Figure 1.

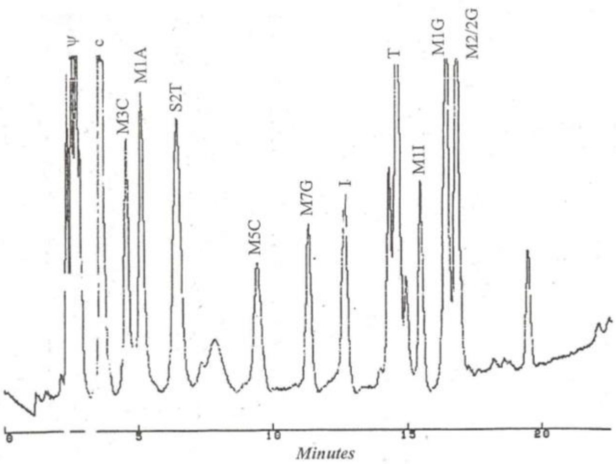

Figure 3 Chromatogram of nucleosides' separation from saliva of breast cancer patients. Chromatographic conditions are the same as in Figure 1.

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