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Determination of Selenium and Vitamin E in Women Breast Milk of Lacting, Commercial and soil by Atomic Absorption Spectrometry and HPLC Respectively

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Abstract

The present work is an attempt to study the level of selenium in (63) samples of human breast milk which were collected in Tikrit region. The concentrations ranged (27.4 - 22.8 mg/L), while the concentration in (143) samples of commercially available infant formula sold in Iraq the concentration was ranged (33.6-22.2 mg/L). From (124) samples animal milk the concentration ranged (22.0- 12.0 mg/L). Selenium content of the samples was determined by atomic absorption – hydride generation. The mean concentration of selenium in the topsoil and subsoil was (0.17, 0.21 mg/kg) , which determined by Graphite Flameless Atomic Absorption spectroscopy. Selenium is an integral part of the enzyme glutathione peroxidase, a long with vitamin E –Se acts as a part of anti-oxidant defense system of cell protecting lipids in cell membranes from the destructive effects of H2O2 and super oxide generated by excess oxygen. in the same mothers, was detected by HPLC, And the level of this vitamin was ranged between (0.82-1.13 µg/dL).

Introduction

Selenium is a mineral that has been officially as essential to human health since the 1980 s. It can also be toxic when consumed at about 14 times the recommended intake. Increasing evidence supports the claim that selenium is an essential dietary micronutrient for human (Picciano,1985).Selenium is of particular interest in infant nutrition because human milk is the only source of this element during the most rapid period of growth. The composition of human milk is believed to provide all the nutrients, including trace elements, necessary for normal infant growth (Perone etal.,1993, Bratter etal.,1997). The selenium level in human milk depends on where the mother lives (Trafikowska etal.,1998). This variation reflects selenium content in the soil and, by the same token, the extent of selenium accumulation in the cereals consumed in a particular region by animals and humans.It is well known that selenium concentration in milk depends on mother's intake of the element (Williams etal.,1983).

Milk from women living in countries where the soil content of selenium is low, such as NewZeland (Kantola etal.,1997), Finland (Tiran etal.,1993), and some other European countries (Levander etal.,1997),has lower selenium concentration compared with milk obtained from women residing in countries with high soil selenium content, such as United States (Hughes and ONG,1998), and Japan (Walivaara etal.,1989).Low blood levels of the selenium have been reported in individuals from several countries, and its deficiency has been associated with several pathological conditions (Salonen etal.,1985). Dietary selenium deficiency in people in certain areas of China is associated with an endemic cardiomayopathy called "Keshan disease", which affects primarily children and women of childbearing age (Chen etal.,1980, Keshan disease research group,1980). In the industries west, dietary selenium deficiency is thought to be associated with cardiovascular disease. Prospective epidemiological study done in Finland revealed the selenium concentration in serum to be inversely related to the risk of cardiovascular disease(Salonen etal,1982, National Research Council,1989).The major source of selenium in most diets is meats and cereal products, which contribute approximately 50%and 25% to 35%, respectively, of total selenium intake of the Americans(Kantola etal.,1997). In addition, dairy products and eggs provide 10% to 20% while fruits and vegetables provide less than 5% of the total selenium intake. For infants and children, however, the core of selenium-providing foods may be much smaller (National Research Council,1989).Farida(Al-Awadi and Srikumar, 2000) and others compared the selenium status in the milk and plasma of Kuwait mothers with that non Kuwait mothers during (0-18) months of lactation .Concentration of selenium in milk and plasma and proteins in milk were significantly higher in the Kuwait group than those of the non Kuwait's during the first 12 months of lactation.L"Abbe(L'Abbe etal.,1996) others determined selenium in infant formula sold in Canada and compared with the selenium content of Canadian breast milk samples, but other study determined selenium, zinc and copper concentration in blood plasma and milk of lacting women from central Poland who where in different stages of lactation and to investigate the relationship between the content of trace element in mothers blood and concentration of microelements in their milk(Wasowicz etal.,2001).

Vitamin E (α – tocopherol) is nature s best fat – soluble (anti-oxidant) in human, its deficiency may cause neurological dysfunction, myopathies and diminished erythrocyte life – span (Campble etal.,1989).

Atomic absorption –hydride generation offer the advantages of high sensitivity and versatility but require all samples to be digested to convert all selenium into the inorganic form. Hydride generation atomic absorption and graphite atomic absorption are widely applied to the determination of Se for concentrations down to 0.05µg /g. The detection limit for GFAAS can be reduced by using stabilized temperature platform furnace (STPF) and by means of Zeeman affect background correction. (Radzium &Thomassen,1992)

Material and Methods

Apparatuses:

The graphite flameless atomic absorption spectrophotometer model shimadzu (P/N 206) was used for the determination of selenium in soil, but hydride generation- atomic absorption spectrophotometer model shimadzu (AA/630) was used for the determination of selenium in milk. Shimadzu liquid chromatography model (LC-6A) equipped with shimadzu detector model (UV- spectrophotometer detector) was used for determination of Vit.E.

Standard solution:

Selenium stock solution (1000mg/L) was obtained by dissolving (0.163gm) in $H_2SO_4$with deionized water and diluting to (1L) final volume. 5% sodium molybdate was prepared by dissolving (5g)of this reagent in deionized water and diluting to 100ml. 1%1,2-diamino -4 nitro benzene prepared by dissolving(0.1gm)of this reagent 1M HCl and diluting to 100ml with HCl.

Selenium analysis in milk: (ALSaleh,2000**)**

Milk selenium content was determined by atomic absorption spectrometry with hydrogen – generation (HG-AAS) with a detection limit of 0.2 mg\L of Selenium, after decomposition of the milk with a mixture of nitric acid and perchloric acid and then reduction of all the Selenium to selenite by boiling with hydrochloric acid, as already described by authors.

Sampling:

The breast milk ,was collected directly in acid –washed polyethylene bottles without the use of a breast pump, after the mother's breast was cleaned by washing with demonized water. All the milk samples after collection were immediately stored indeep freezer at (- 18 C^o) until selenium analysis.

Selenium analysis in soil :(Xiao-quan etal.,1985**)**

Weigh 0.25 to 1.00 gm of air-dried soil sample was weighed and ground to pass through 200µm sieve to a100 ml conical flask. 0.2 gm of ascorbic acid was added and adequate volume of deionized water to wet the soil sample thoroughly. 1.0 ml of5% sodium molybdate was added to aqueous solution and 7.0 ml of (3+4) mixture of sulfuric acid and perchloric acid. Gently heat the sample at120Cº on a hot plat for12-20 min ,then raise the temperature to about 160°C until the digest turns yellowish-green and white silica begins to form .The flask was removed from the hot plate and when cool, wash the inside wall of the flask with about 10 ml of deionized water. Filter the soil digest using afine- porosity filter paper in to a100ml separatory funnel and wash the silica with deionized water several times. The total volume of filtrate and washing should be about 35ml.Remove the filter funnel add 1ml of 1%1,2-diamino-4 nitro benzene solution 1M hydrochloric acid, mix well and let stand for 1hr.Extract the 5-nitro .2, 1, 3-benzoselenadiazole into a 5 ml of chloroform by shaking the mixture for 2 min. After the phase separation, transfer the organic layer into a10 ml quartz centrifuge tube and cover the tube with a quartz stopper .Inject 20 ml of organic extract in to the graphite tube .dry at 100ºC for 30 sec, char at 200 Cº for 30 second atomize at 2200Cº for 4 sec.Measure the absorbance of selenium at its resonance line of 196.0 nm under thestopped flow condition.

Vit.E- determination :(Jansson etal.,1998)

Serum fat soluble vitamin E was analyzed by isocratic reversed-phase liquid chromatography with diode array detection (11-13) separations were performed using a 250mm × 4.6mm Varian Octadecyl silane C-18 column packed with 5µm Particular at 35C^{o} .The mobile phase methanol (HPLC grade) was pumped at 0.8 ml/min and detection was performed by scanning from 240-360 nm. The optimum detection wave-length was 325nm and 290nm corresponding to the absorption maximum for (α-tocopherol)**.**

Results

Table (1): show the accuracy and precision of the method graphite flameless –atomic absorption method for determination selenium

Se added **mg.l^{-1}**	Se found **mg.l^{-1}**	% recovery
0.055	0.059	93.32
0.081	0.087	93.10
0.110	0.114	96.49
0.21	0.213	98.59

Se lower detection limit =0.0086 mg/L
Correlation coefficient =0.999

Table (2): show the accuracy and precision of the method hydride generation–atomic absorption method for determination selenium

Se added **mg.l^{-1}**	Se found **mg.l^{-1}**	% recovery
0.95	0.97	98.0
1.08	1.09	99.0
1.21	1.21	100.0
1.34	1.35	99.2

Se detection limit =0017 mg/L
Correlation coefficient = 0.998

Table (3): Show the accuracy and precision of (HPLC method) for determination Vit.E

Se added **mg.l^{-1}**	Vit E foundMg/dL	% Recovery
0.25	-	-
0.55	0.56	98.2
0.87	0.85	102
1.81	1.82	99.45

Vit E lower detection limit =0.0050 mg/L
Correlation coefficient = 0.999

Table (4): Mean selenium breast milk concentration (mg/L).

mg/L	groups	No. samples
27.4(33.6-21.8)	18-25 years	25
24.07(40.2-21.3)	26-35 years	18
22.8(34.1-17.5)	36-45 years	20

Table (5): Selenium content in commercially available infant formula sold in Iraq.

mg/L	types	No. samples
29.2 (27-32)	guigoz (1)	35
33.0(34.1-36.2)	guigoz (2)	27
22.2(27.7-33.0)	pelargon	22
26.6(24.0-32.0)	Bebelac	33
32.4 (33.3-29.1)	cemelac	26

Table (6): selenium concentration in animal milk.

mg/L	types	No. samples
22.0 (14.2-25.0)	Cow	36
18.0 (5.3-21.8)	Sheep	24
12.0 (10.0-18.9)	Goat	27

Table (7): Determination of vita. E in whole blood of the women which determined selenium in the breast milk in table -1-

Mg/L	groups	No. samples
1.43(2.01-1.7)	20-38 years	Control
1.13 (1.7-0.8)	18-25 years	25
0.93 (1.2-0.7)	26-35 years	18
0.82 (1.1-0.5)	36-45 years	20

Discussion

It is well known that several areas in the world have low selenium content in the soil (Kantola etal.,1997) and that this is responsible for the low dietary intake of the element (Tiran et al.,1993).The level of selenium in the soil influence its amount in foodstuffs and thus the dietary selenium intake, consequently, its level in milk (Kantola etal.,1997, Hughes and ONG,1998).The marked geographic differences in selenium level in human milk are reflected in the differences in dietary Se intake by breast-fed infants (Tiran etal.,1993, Levander etal.,1997).In adequate Se intake by lactation mothers can be increased by supplementation of inorganic or organic selenium (Bratter etal.,1997, Trafikowska etal.,1998, Tiran etal.,1993).The mean concentration of selenium in the topsoil and subsoil was (0.17 ±0.089 mg/kg) from (42) samples and (0.21± 0.07 mg/kg) from (38) samples contents respectively. The values correspond to the average total selenium content in the soil of the low Se keshan disease in china (0.112mg/kg) range (0.059-0.10 mg/kg) (92),other low values have also

been in the world reported such as (0.198mg/kg) and (0.23mg/kg) from Yugoslavia (Jovic,1998, Ma KSIMOVIC .Z etal,1992) less than 0.1mg/kg from Newzeland, Hungary and Finland(Westermark etal.,1977, Gondi etal.,1992). The low selenium content Tikrit area may be attributed to the nature of the soil in the area studied which is a well –drained, non saline to moderately saline, calcareous, Loamy soils with sandy topsoil.

Table (4) shows the concentration of selenium in women breast milk from Tikrit area, the level of selenium concentration is normal between ages (18-25) years, but it ranged from ages (26-45) years, which the concentration ranged (27.4-22.8 mg/L) the Se levels in mature milk are much higher although, the results of this study show uniform distribution of Se in human milk when comparable with the selenium levels in milk in some other European countries, such as German (Rubberiest etal,1985), Sweden (Walivaara etal.,1989) and Finland (Westermark etal.,1977). In these countries in Europe (Trafikowska etal.,1998, Keshan disease research group,1980, Salonen et al.,1982, National Research Council,1989).

The selenium intake of infants is of interest because of their rapid growth rate and their heavy reliance on milk, a food that has a highly variable Se content, depending on its geographical origin. At 6 months of age, milk provided only 35%, whereas cereals provided 34% of the total dietary Se. By 12 months of age, the introduction of a variety of foods further decreased the relative contribution of milk and cereal to the Se nutrition of the infants (Jochum&Fuchs,1995). Se is an important element which enter in the composition of some enzymes which known as selenoenzymes, as Iodothyronine -5- denominate (ID-I) which is present in the brain, pituitary & placenta. (Walivaara et al.,1989) These enzymes are responsible for the growth of the babies' brain and baby bodies while the deficiency of selenium concentration in diet will inhibits the enzyme activity (Bratter etal.,1997). There is a good correlation between plasma selenium content and plasma glutathione peroxides, in which selenium is an important part of this enzyme, and it protect cells against the effects of free radicals that are produced during oxygen metabolism (Hughes and ONG,1998).Low selenium level in the blood of women at preterm delivery might be one of the cause of retinopathy and respiratory distress syndrome (Walivaara etal.,1989).Table (5) shows the selenium content in commercially available infant formula solid in Iraq, the differences in the level of selenium concentration range between (22.2-33.0 mg/L) which is used it to increase the level of this element in the children body .

Selenium concentration in formula milk is generally lower than in breast milk (Jochum&Fuchs,1995).The selenium status of bottle-fed children depends on the Se level in milk substitutes.Plasma selenium concentration and red blood cell glutathione peroxides activity levels of newborn infants are lower than those of their mother or other adults (Salonen etal.,1985), and due to low concentration of selenium in the plasma of new born breast milk or dried milk will reduce this effect(Trafikowska etal.,1998) and table (6) shows the concentration of mean selenium content in breast milk of(Goats,Sheeps and Cows) the concentration was ranged (12.0-22.0 mg/L) the level of this element in breast milk of dependent partially on Se content in grass and in plants . From the results concluded that this region is suffered from selenium deficiency.Table (7) shows the range, of vitamin E in the whole blood of same women from table (4), the range of this vitamin was between (1.13-0.82 µg/dL) this at low level when compared with the control status because they suffering from low concentration of selenium and this vitamin act as a part of anti-oxidant defense system of cell protecting lipids in cell membrance from the destructive effects of H_2O_2 and superoxide generated by excess oxygen absorption.

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تقدير فيتامين E والسلينيوم في الحليب والتربة والحليب التجاري بواسطة الامتصاص الذري

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الخلاصة

تـــم في هذا البحث قياس مستوى السيلينيوم في (٦٣) نموذج من حليب الأمهات في محافظة تكريت ، وبأعمار تراوحت بين (١٨- ٤٥) سنة ، وكان التركيز (٢٢,٨-٢٧,٤ ملي غرام/لتر) ، وأيضا تم قياس التركيز في الحليب المجفف المستورد، فكان التركيز (٢٢,٢-٣٣,٦ ملي غرام/لتر)، وبلغ مستوى تركيز العنصر لأكثر من (٧٧) نموذج من حليب البقر والماعز والأغنام وكان التركيز للأنواع الثلاث (٢٢,٠-١٢,٠ ملي غرام/لتر) وتم قياس عنصر السيلينيوم في العينات المذكورة أعلاه بالامتصاص الذري- توليد الهيدريد ، أما مستوى السيلينيوم في سطح وتحت التربة (٠,٢١ ، ٠,١٧ كيلو غرام/ملي غرام) وباستخدام مطيافية الامتصاص الذري غير اللهبي .كما أمكن قياس مستوى فيتامين(E) الذي يعد السيلينيوم من أهم مكونات الفيتامين ويعملان كمضادات للدفاع عن الخلايا وكان التركيز (١,١٣- ٠,٨٢ دسي لتر/مايكرو غرام) و تم القياس بواسطة كروماتوغرافيا السائل عالي الأداء.