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# ASSESMENT OF SELENIUM STATUS OF HEALTHY ADULTS IN SOUTH WESTERN NIGERIA

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## ABSTRACT

Blood selenium (BSe) and plasma glutathione peroxidase (plGSH-Px) activity were measured as biochemical markers of selenium status of 88 apparently healthy subjects (40 males; 48 females). The subjects were recruited from Abeokuta and Ibadan (South-Western Nigeria).

The mean age of the healthy subjects was 37.8 ± 8.6 (range 18 - 52) years. Their weight and height were measured and their body mass indices (BMI) computed. BSe was determined by atomic absorption spectrophotometry (AAS) while plGSHPx activity was measured by spectrophotometric method.

The mean BSe concentration in healthy adults was found to be 0.188 + 0.026 mg/L while the mean plGSH-Px activity was 0.127 ± 0.022 U/mL. These values were compared with some of those that were reported from other parts of the world.

It is evident from these results that there is no evidence of selenium deficiency in this environment since the range of BSe values found in healthy subjects is comparable to those found in similar populations in many areas of the world where selenium concentration in the soil and in human diet is considered to be adequate.

Key Words: Blood Selenium, Glutathion peroxidase, Selenium status, Biochemical markers

## INTRODUCTION

Until recently the only known metabolic role for selenium in mammals was as a component of the antioxidant enzyme glutathione peroxidase (GSH-Px)(EC 1.11.1.9). However, selenoproteins other than GSH-Px have now been found in tissues of several species (Lockitch,1989).

Selenium was found to be associated with a protein in the tail of spermatozoa from bulks and rats (Calvin *et al.,* 1987), thereby establishing a specific requirement for selenium in spermatogenesis in these species.

Selenium was also found as an integral component of a protein - Type 1 iodothyronine 5' deiodinase - which activates thyroxine to triiodothyronine, thus selenium plays an indispensable role in thyroid hormone synthesis (Zagrodzki *et al.,* 2000).

Burk, Hill, Read and Bellew (1991) also purified and characterized a selenoprotein known as selenoprotein P from rat and human plasma, though its function in human physiology is not yet known. However selenoprotein P was hypothesized to be a selenium-transport protein (Hill and Burk, 1994), though there was no convincing evidence to support this claim (Arthur and Beckett, 1994).

Additionally, the discovery that selenium could prevent liver necrosis in vitamin E deficient rats (Schwarz and Foltz, 1957), pancreatic atrophy in vitamin E

supplemented chicken (Thompson and Scott, 1969) growth retardation, hair loss and cataract in vitamin E replete rats (McCoy and Weswig, 1969) confirmed its nutritional essentiality.

The present study was therefore designed to determine the blood selenium concentration (BSe) and glutathione peroxidase (GSH-Px) activity in plasma of apparently healthy adults in south western Nigeria as indicators of their selenium status. As far as we are aware there are no reports on selenium status of people in this environment.

## MATERIALS AND METHODS

*Selection of subjects*

These consist of eighty-eight apparently healthy subjects of both sexes, aged between 18 and 52 years. They were all non-smokers and non-alcoholics and were mainly students, office workers and traders. They were not taking any medication during the period of the study. They were also classified into male and female subgroup. Informed consent was obtained from all participants after being educated on the benefit of the study.

*Anthropometric indices*

The current ages of the subject were noted. Also the current body weight were measured with minimal clothing using a balance beam scale. Heights were also measured barefooted, using a meter rule. Height (m) and Weight (Kg) were used to calculate the body mass index (BMI) (kg/$m^2$).

*Collection of blood*

About 10ml of venous blood was obtained from the antecubital fossa vein using disposable pyrogen-free needle and syringes (Beckton-Dickinson, Dublin, Ireland). About 3ml of blood was dispensed into heparinized tubes containing lithium heparin (Vacutainer system Inc.. Rutherford, New Jersey), this was frozen as heparinized whole blood sample. The remaining blood sample was dispensed into plain vacutainer tubes containing EDTA (Sterilin Manufacturing Co, England) to prevent coagulation. The samples were centrifuged at 450 g in IEC centrifuge (Ames Technicon, France) for about 5mins to obtain plasma, which was transferred with a pipette into plain vacutainer tubes and then frozen. Samples were kept frozen at -70°C until analysed.

GSH-Px activity was assayed by a

*Determination of plasma glutathione peroxidase (EC 1.1.11.9)*

modification of the . couple method of Paglia and Valentine (1967) using Hydrogen peroxide as substrate and monitoring the oxidation of NADPH at 340nm. One unit of enzyme activity was defined as I mole NADPH oxidized/min. and the result is expressed as unit/ml of plasma.

*Determination of blood selenium*

Selenium in blood was determined with atomic absorption spectrophotometer (AAS) by the method of Pleban, Munyani and Beachum (1982) using Perkin Elmer AAS model 703 (Perkin - Elmer Oak Brown, Illinois, USA) equipped with AS 60 automatic sampler and a selenium hollow cathode lamp.

The blood samples were first thawed (make liquid after being frozen) and diluted (1:2) with a 0.1% nitric acid solution containing

0.1% Triton X-100 (Skybio Ltd, Wyboston, Bedfordshoe, UK). This solution was then mixed thoroughly and about 0.2m1 aspirated into AAS for analysis.

*Quality control*

Pooled human blood was used to assess the quality of the selenium measurements. The mean analyzed value (+ SD) was 0.183 + 0.0042 mg/L and within batch precision was 2.3% (CV)

*Determination of urea in plasma*

Urea concentration in the plasma was determined by the method of Coulombe and Favreau (1963)

*Determination of creatinine in plasma*

Creatinine concentration in the plasma was determined by the method of Taussky (1956)

*Determination of uric acid in Plasma*

Uric acid concentration in the plasma was determined by the method of Henry, Sobel and Kim (1957)

*Determination of sodium and potassium in plasma*

Sodium and Potassium concentration in the plasma was determined by Flame Photometry

*Statistical Analysis*

Results were expressed as mean + standard deviation (SD). Student t-test was used to determine significance between means at 5% level of significance.

## RESULTS

Table 1 shows the anthropometric indices of the healthy subjects. The mean age of

Table 1. Age, weight, height and body mass index of healthy subjects

| | *Healthy Subjects (n = 88)* | *Healthy Males (n = 40)* | *Healthy Females (n = 48)__ ,* | |
|---|---|---|---|---|
| Age (years) | 37.8 ± 8.6 (18-52) | 35.4 ± 8.3 | N 37.9 + 9.5 | NS |
| Weight (kg) | 62.2 ±9.3 (42-78) | 62.9 ± 9.2 | 61.5 ± 9.3 | NS |
| Height (metres) | 1.66 X0.09 (1.45 - 1.80) | 1.71 ± 0.09 | 1.64 ± 0.09 | ** |
| Body Mass Index BMI (kg/m$^2$) | i 21.06 ± 2.74 (15.57 - 26.70) | 20.14 ± 2.06 | 21.22 ± 1.78 | |

P'= Comparison of healthy male and female
Values are in mean ± SD n = No. of Subjects
BMI = Body Mass Index
SD = Standard Deviation

Table 2. BSe and pIGSH-Px activity in healthy subjects

| | Healthy Subjects (n = 88) | ( males Healthy (n = 40) | Healthy females (n = 48) | |
|---|---|---|---|---|
| BSe (mg/L) | 0.188 + 0.026 | 0.190 ± 0.028 | 0.185 ± 0.025 | |
| pIGSH-Px (U/ml) | 0.127 + 0.022 | 0.129 + 0.029 | 0.127 + 0.026 | NS |

P' = Comparison of healthy male and female
Values are in mean ± SD BSe = Blood selenium
pIGSH-Px = plasma glutathione activity
NS = No significant difference n = No of subjects
SD = Standard deviation * = P<0.05

healthy group (n = 88) was 37.8 (SD 8.6; range 18 - 52) years . Their mean weight, height and Body mass index (BMI) were 62.2 (SD 9.3; range 42 - 78) kg, 1.66 (SD 0.09; range 1.45 - 1.80) m and 21.06 (SD 2.74; range 15.57 - 26.70) $kg/m^2$ respectively.

There were no significant differences between the healthy male and healthy female with respect to age and weight, however there were significant differences in their height and BMI ($P < 0.01$ and $P < 0.05$ respectively).

Table 2 shows the mean level of BSe and pIGSH-Px in healthy subjects. There was no significant difference in the pIGSH-Px level of the healthy male and healthy female, however there was a significant difference in their BSe level.

Table 3 shows the mean values of total protein, albumin, creatinine, urea, uric acid, sodium and potassium in healthy subjects as well as the mean values of these biochemical parameters in healthy males and healthy females. The mean values of total protein and uric acid were significantly higher in healthy males than in healthy females ($P < 0.001$), whereas the mean values of albumin and plasma $Na^+$ were significantly higher in healthy females than in healthy males ($P < 0.001$). The mean values of creatinine, urea and plasma $K^+$ were similar in both groups.

Tables 4 shows the BSe values in healthy free living population of different areas around the world. The values were compared with those obtained in this study.

## DISCUSSION

A wide spectrum of reference ranges for blood selenium in healthy adult populations has been reported from different countries. However, thus far there are no data available for selenium status of the general populations in many other countries and regions of the world including Nigeria. The

Table 3. Plasma total protein, albumin, total globulin, creatinine, urea, uric acid, sodium and potassium in healthy subjects

| | Healthy Subjects (n = 88) | Healthy males (n = 40) | Healthy females (n = 48) | |
|---|---|---|---|---|
| Total Protein (g/L) | 76.1 ± 7.3 (61.3 - 85.6) | 76.4 ± 4.1 | 74.2 ± 4.5 | |
| Albumin (g/L) | 46.1 ± 10.2 (33.3 - 59.7) | 43.2 ± 8.4 | 49.3 ± 6.6 | *** |
| Creatinine (mmol/L) | 62.5 + 19.9 (26.5- 114.9 | 63.2 ± 10.1 | 63.1 ± 14.2 | NS |
| Urea (mmol/L) | 4.4 ± 1.6 (2.3-7.3) | 4.8 + 0.8 | 4.3 ± 0.9 | NS |
| Uric acid (mg/L) | 43.2 + 2.8 (31.8 - 49.8) | 49.2 + 4.2 | 43.8 ± 4.0 | *** |
| $Na^+$ (mmoUL) | 137.3 ± 2.0 (135-140) | 134.3 ± 2.0 | 137.8 ± 2.0 | *** |
| $K^+$(mmol/L) | 4.9 ± 0.5 (3.8-5.2) | 4.9 ± 0.5 | 4.8 ± 0.4 | NS |

P'= Comparison of healthy male and female
Values are in mean ± SD
NS = No significant difference N = No of Subjects $Na^+$ = Sodium ion $K^+$ = Potassium ion *** = P<0.001
** = P<0.01 * = P<0.05

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present study is probably the first attempt at obtaining a baseline value for blood selenium in this environment and comparing this with values that were reported from other parts of the world.

For this study, whole blood selenium (BSe) and plasma glutathione peroxidase (p1GSH-Px)(indices of selenium status) showed wide range of levels in the healthy group. These ranges probably reflect the wide range of selenium intake. The mean level of BSe in healthy adults was 0.188 ± 0.026 mg/L while the mean value of p1GSH-Px activity of this group was 0.127 ± 0.022 U/mL.

The lowest mean values of BSe so far of 0.056 ± 0.017 mg/L and 0.059 ± 0.012 mg/L have been reported from Lappeenranta, Finland (Westermack *et al.*, 1977) and Dunedin, New Zealand (Rea *et al* 1979) respectively; while the highest mean value of 3.2 + 1.300 mg/L was

Table 4. Reported values for BSe concentration for healthy adult populations from different parts of the world

| Research Group | Country/Region | Selenium Concentration Mean (± ISD)(mg/L) |
|---|---|---|
| Present Study | Western Nigeria j (Abeokuta & Ibadan ) | 0.188 + 0.026 |
| Bowen & Cawse (1963) | United Kingdom | 0.320 |
| Brune *et al.* (1966) | Sweden | 0.120+ 0.02 |
| Burk *et al.* (1967) | Guatemala | 0.230+0.05 |
| Dickson & Tomlinson (1967) | Canada, Ontario | 0.182 + 0.036 |
| Allawas *et al.* (1968) | USA, South Dakota | 0.256 + 0.036* |
| Allawas *et al.* (1968) | USA, Ohio | 0.157 ± 0.032* |
| Suchkov(1971) | USSR, Ukrainian SSR | 0.442 ± 0.034* |
| Maxia *et al.* (1972) | Egypt | 0.068 |
| Abdullaer (1976) | USSR, Azerbeijan SSR | 0.110 + 0.007 |
| Westermarck *et al.* (1977) | Finland, Helsinka | 0.081 + 0.015 |
| Westermarck *et al.* (1977) | Finland, Lappeenranta | 0.056 + 0.017 |
| Rea *et al.* (1979) | New Zealand, Dunedin | 0.059 ± 0.012 |
| Robinson & Thomson (1981) | New Zealand, Auckland | 0.083 ± 0.013 |
| Ward *et al.* (1982) | Scotland | 0.199 + 0.017 |
| Yang *et al.* (1983) | China, Beijing (Moderate Selenium area) | 0.095 ± 0.091 |
| Yang *et al.* (1983) | China, High-Selenium area with history of intoxication | 3.2+ 1.300 |
| Yang *et al.* (1983) | China, Low- Selenium area without Keshan disease | 0.027 + 0.009 |
| Yang *et al.* (1983) | China, Low -Selenium area with Keshan disease | 0.021+ 0.010 |
| Spangno to *et al.* (1991) | Italy | 0.105 ± 0.012 |
| Popadiuk *et al.* (1995) | Poland | **0.086** ± 0.019 |
| Cser *et al.* (1995) | Germany | 0.089 ± 0.017 |
| Cser *et al.* (1996) | Hungary | 0.064 ± 0.011 |

reported from China in high selenium area where there is history of intoxication (Yang *et al., 1983).*

The mean BSe value of healthy adults obtained in this study is comparable with those obtained from Ontario, Canada (Dickson and Tomlinson, *1967),* Ohio, USA (Allaway *et al., 1968)* and Azerbeijan, USSR (Abdullaev, *1976).* The value is slightly higher than those of Scotland (Ward *et al., 1982)* and Italy (Spangnolo *ei al., 1991)* This value is however different from those obtained from South Dakota, USA (Allaway *et al., 1968)* Ukrainian and Auckland, New Zealand (Robinson and Thomson *1981).* This is not unexpected however, because of the w 'de variation in selenium content of foods from different regions around the wc'ri;i.

Comparative environmental studies in soils and foods and the selenium status of the inhabitants carried out in China alone showed that the distribution of selenium is such that five types of areas have been identified (Yang, Wang, Zhou and Sun, *1983).* These ranged from seleniferous areas in which clinical selenosis occurred in livestock and humans, through seleniferous areas where no clinical selenosis was seen, areas of adequate selenium content, areas of low selenium content, and areas in which extremely low selenium content were associated with clinical syndromes of selenium deficiency.

Furthermore analytical survey on humans has confirmed that the significant variation in BSe level in different geographical areas, is presumably as a result of differences in dietary selenium. For example Allaway *et al. (1968)* found that selenium levels in the blood of persons living in areas of the USA where soils and vegetation were high in selenium tended to be higher than that of persons living in areas where soils and plants were generally low in selenium. Thus geographical differences in selenium level in human blood are possible, even in countries where large-scale interregional food shipment would be expected to level out the differences in the amount of selenium in the food supply (Lockitch, *1989).*

These geographical differences in human BSe level was also confirmed in the USA by Howe *(1974),* who found a mean BSe level of *0.265* mg/L (+ *0.056;* SD) for *626* samples collected in and around the state of South Dakota (a high soil selenium region) whereas Schultz and Leklem *(1983)* reported a mean BSe level in Oregon (a low soil-selenium region) that were well below those reported for other areas of the USA.

Consequently, the mean BSe level in healthy adult population in this study probably reflects the level of dietary selenium in this environment, and since the value is comparable to those obtained in parts of the world where blood selenium level is said to be adequate, this probably suggests that there is no selenium deficiency in this environment.

Many factors that are known to affect blood selenium assessment in humans apart from the influence of diet and geographic environment were carefully identified in this study and well taken care of, to ensure that the measured values of BSe is the true reflection of the selenium status of the healthy adults in this environment.

For example, selenium levels in healthy adults were known to be altered by alcohol, tobacco, or oral contraceptive use ( Moore *et al., 1984).*

A strong inverse relationship between blood selenium and smoking was shown in several studies (Lockitch, 1989). Serum selenium was inversely correlated with number of years of smoking (Moore, Noiva and Wells, 1984), and was lower in smokers who drank alcohol (Lloyd, Lloyd and Clayton, 1983). This is the reason all participants in this study were non-smokers and non-alcoholics.

Other factors such as age, sex, and lifestyle habit have also been considered. Oster, Prellwitz, Kasper and Meinertz (1983) had shown that BSe concentrations were similar for men and women. The findings in this study however were not in agreement with this view. The mean value of BSe in healthy males was significantly higher than that of healthy females($P<0.05$). The difference may probably reflect hormonal influence or previous losses through breast milk, because it was known that mothers that breastfeed their infant exclusively have lower BSe level (Kumpulainen *et al.,* 1985).

Plasma Glutathione peroxidase is also a useful index for assessing human selenium status, because it represents a functional form of selenium (Lockitch 1989). It is measured as the second index of selenium status of the healthy subjects in this study.

The mean plasma GSH-Px activity obtained in this study is similar to those of Thompson *et al.* (1977) and Pleban *et al.* (1982). However, Zagrodzki *et al.* (2000) observed that comparison of results of plasma GSH-Px activity from different laboratories has limited meaning because of the lack of standardized methods used for he determination of plasma GSH-Px activity.

There are many other difficulties and limitations that have been associated with the determination of GSH-Px activity. For example, the enzyme is not stable and hence its activity cannot be determined in samples stored for a very long time, whereas measurement of selenium concentration can be made on stored samples (Lawrence and Burk, 1976). This is the reason the determination of p1GSHPx was carried out promptly.

Another confounding factor in the use of GSH-Px to assess selenium status is the existence of a "non-selenium dependent" GSH-Px activity in some tissue e.g. testis (Lawrence and Burk, 1978), so also in rat brain, kidneys, liver and adrenals. No such activity was found in the blood, spleen, heart and skeletal muscle (Lawrence and Burk, 1978). This non-selenium containing enzyme has been shown to differ from selenium containing enzyme in substrate specificity in that it catalyses the reduction of organic hydroperoxides but not hydrogen peroxide (Lawrence and Burk, 1976).

Obviously, any use of GSH-Px to monitor selenium status must take into account this 'non-selenium dependent' activity. This complication can be avoided by selecting a tissue for test that has little or none of the selenium independent activity such as plasma or erythrocytes or by using hydrogen peroxide as the substrate, since this has little activity with the selenium independent enzyme. These were well taken care of by the method of Paglia and Valentine (1967) adopted for this study. Plasma was asp'^sed and hydrogen peroxide was used as substrate.

The mean levels of plasma total protein, albumin, urea, uric acid, creatinine, $Na^+$ and $K^+$ in healthy subjects all fall within the normal range for healthy adults in this

environment. This is also true for these biochemical parameters in healthy males and healthy females separately. Consequently, this study provides a reliable scientific data for selenium status of apparently healthy adult population in this environment.

## REFERENCES

Abdullaev G. M, 1976. Selenium levels in healthy subjects and those with certain haematological diseases. In : *Selenium Biology,* Baku, Elm Publishing House (in Russian) Vol.1, pp 136-139

Allaway, W. H., Kubota, J., Losee, F., and Roth, M. 1968. Selenium, Molybdenum, and Vanadium in human blood. *Arch. Environ. Health,* 16: 342-348.

Arthur, J. R. and Beckett, G. J. 1994. Newer aspect of micronutrients in at risk Groups: New metabolic role for selenium. *Proc. Nut. Soc.* 53: 615 - 624.

Burk R.F., Hill K.E, Read R and Bellow T 1991. Response of rat selenoprotein -P to selenium administration and fate of its selenium. Am. J. Physiol. 261: 26 - 30

Calvin, H. L, Grosshans K., Musicant - Shikora, S. R. and Turner, S. 1.1987. A developmental study of ratsperm and testis selenoproteins. *J. Reprod Fert.* 81, 1-7.

Coulombe J. S, and Favreau L.A 1963. A simple colorimetric method for the determination of urea in the presence of diacetyl monoxime and thiosemicarbazide. *Clin. Chem. 9 :* 102 -104.

Dickson, R.C. and Tomlinson, R.H. 1967. Selenium in blood and human tissues. *Clin.Chim. Acta,* 16: 311-321.

Henry R. J., Sobel S and Kim J. 1957. A phosphotungistic acid method for the determination of uric acid in biological fluids. *Amer. J. Clin. Path.* 28: 152 - 154

Hill K. E and Burk R. F 1994. Selenoprotein P- an extra cellular protein containing multiple selenocysteins. In selenium in Biology And Human Health (R.F Burk, Editor). New York : Springer Verlag pp 117-131

Howe, M. 1974. Selenium in the blood of South Dakotans. *Arch. Environ. Health* 34:444 - 448.

lloyd, B. Lloyd, R. S., and Clayton, B. E. , 1983. Effect of Smokings alcohol and other factors on the selenium status of a healthy population, J. *Epidenniol, Commun. Health 57, 213.*

Kumpulainen J., Vuori E. and Siimes M. A., 1985. Effect of maternal dietary selenium intake on selenium level in breast milk. *Int. J. Vitamin Nun•. Res.* 54 , 251-253.

Lawrence, R.A. and Burk, R.F. 1978. Species, tissue, and subcellular distribution of non Se dependent glutathione peroxidase activity. *J. Nutr.,* 108:21 1-215.

Lawrence, R. A. and Burk, R. F.1976. Glutathione. Peroxidase activity in selenium deficiency rat liver, *Biochem. Biophys. Res. Commun.* 71, 952-558.

Lockitch G. 1989. Selenium, clinical significance and analytical concepts *Cri. Rev.Clin.* Lab. Sc. 27, 483-542.

McCoy, K.E. M., and Weswig, P. H.1969. Some selenium responses in rat not related to Vitamin E. *J Nut.* 98,383-390.

Moore, J.A., Noiva, R., and Wells, I.C. 1984. Selenium concentrations in plasma of patients with arteriographically defined coronary artherosclerosis. *Clin. Chem.,* 30(7): 1171-1173.

Oster, O. Prellwitz, W.; Kasper, W and Meinertz, T.1983. Congestive cardiomyopathy and selenium content of serum. *Clin. Chim. Acta.* 128, 125-132.

Paglia D. and Valentine W'.N. 1967. Study on the quantitative and qualitative characterization of erythrocyte glutathione peroxides *J. Lab. Clin Med.* 70 154 -169

Pleban, P.A., Munyani, A., and Beachum, J. 1982. Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes, 28:311-3 *16. Clin. Chem*

Rea, H.M., Thomson, C.D., Campbell, D.R., and Robinson, M.F. 1979. Relation between blood selenium concentrations and glutathione peroxidase (EC 1.11. 1.9) activities of New Zealand residents

and visitors to New Zealand. *Br. J. Nutr., 42: 201208.*

Robinson, and Thompson, *1981.* Selenium levels in humans vs environmental sources. Selenium in Biology and Medicine, Westport, Conneticut, The AVI Publishing Co. Inc. *pp 283-302.*

Schultz, T.D. and Leklem, J. E. *1983.* Selenium status of vegetarians, nonvegetarians, and hormonedependent cancer subjects. *Am. J. Clin. Nuir., 37: 114-118.*

Schwarz, K. and Foltz, C. M. *1957.* Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc., 79: 3292-3293.*

Spangnolo, A., Morisi, G., Marano, G., Righetti, G., Masetta, A. Menotti, A. *1991.* Bloodselenium and precursor of cardiovascular risk factor in *adolescents Eur J. Epidemiol 7: 654 - 657.*

Taussky H.H *1956.* A modified alkaline picrate method of determination of creatinine in serum. *Clin. Chim. Acta. 1: 200-202*

Thompson, J. N. and Scott, M. L. *1969.* Role of selenium in the nutrition of the chick. J. Nutr., *97: 335-342.*

Thomson C. D. Rea, H. M., Doesburg V. M. and Robinson M. F. *1977.* Selenium concentration and glutathione peroxidase activities in whole blood of New Zealand residents *Br. J. Nutr., 37 457-460*

Ward, K, Anderson. J. Arthur, J. R., Aggett, P. J. *1982.* Selenium status in healthy and disease states. *Proc. Nutr. Soc. 41: 20-23.*

Wastermarck, T., Raunu, P., Kirjarinta, M., and Lappalainen, L. *1. 1977.* Selenium content of whole blood and serum in adults and children of different ages from different parts of Finland, *Acta. Pharmucol, Toxicol. 40, 465-475.*

Yang, G., Wang, Z., Zhou, R. and Sun, S.I. *1983.* Endemic selenium intoxication of human in China *Am. J.Clin. Nutr. 37, 872-876.*

Zagrodzki A. D., Szmigiel, H., Ratajczak, R.. Szybinski, Z. and Zachwiega, Z. *.2000.* The role of selenium in iodine metabolism in children with goiter. *Environmental Health Perspectives 108, (I), 67-71.*