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Determination of Selenium Concentration in Serum and Toenail as an Indicator of Selenium Status

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Selenium is a naturally occurring element in the environment and is an essential trace element in humans. The key role of selenium in mammalian metabolism is attributed to the presence of four selenocysteine residues in the enzyme glutathione perioxidase. This enzyme catalyzes hydrogen peroxide and lipid peroxides and thus contributes to the protection of membrane lipids and other cellular and extracellular components from oxidative damage (Chow 1979).

Selenium deficiency have been reported in individuals from several countries. It has been associated with several pathological conditions such as cancer, infectious/inflammatory diseases and immune system (Ryan-Harshman and Aldoori 2005). Selenium content of foods is largely related to the selenium content of soil (Combs 2001). Available data show that the selenium intake of inhibtants in different regions is widely variable, depending on differences in dietary habits and regional geobotanical selenium status (Robberecht et al. 1994).

In most studies, selenium status is; usually, assessed by measuring it in serum or plasma, erythrocytes, platelets (Campbell et al. 1989) or whole blood, and by the determination of whole blood glutathione peroxidase activity. Serum selenium is influenced by eating habits and does not reflect long-term exposure to selenium (Levander 1985), whereas erythrocytes accumulate selenium and presumably reflect intake over their 120-day life span (Rea et al. 1979). Glutathione peroxidase activity in the blood is closely related to the blood selenium levels. It is thought to be functional parameter of the selenium status, as it is depressed in selenium deficiency. There have a number of investigators demonstrated the feasibility of assessing selenium in toenail as it displays long-term indicator of selenium status (Garland et al. 1993; Krogh et al. 2003) and reflects differences in selenium intake (Hunter et al. 1990). The time period, which is reflected by toenails, cannot be specified precisely. Toenails grow 1 mm per month and a normal toenail takes 12 to 18 months to grow completely (Fleckman 1985). Thus, toenail clippings reflect a time period of 12 to 18 months. Our previous study (Al-Saleh et al. 1999) found that the selenium content in the topsoil and subsoils of Al-Kharj District were close to the average total selenium content in the soil of low-selenium Keshan disease areas in china (Sun et al. 1985). These observations

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led us to conclude that low selenium-status in soil might be reflected on dietary selenium-intake level by the Saudi population living in Al-Kharj District. From 2003 to 2004, we conducted a cross sectional study assessing the selenium status of subjects living in the area. This has explored a good opportunity to compare the usefulness of measuring selenium in serum and toenail samples in assessing selenium status. The analytical methodology used in this study has been found to be effective in determining selenium concentrations in the ppb levels.

MATERIALS AND METHODS

Selenium reference solution (1000 PPM) and 30% hydrogen peroxide were obtained from Fisher ChemAlert Guide, Fisher Scientific Co. Trace metal free hydrochloric acid and "selectipur" nitric acid select were obtained from Fisher Scientific Co., Pittsburgh, PA, USA, and E. Merck, D-6100 Darmstaot, Frankfurter Strasse 250 Germany respectively. Sodium hydroxide was bought from Berck Spencer Acid Ltd and sodium borohydride was obtained from BDH Chemicals Ltd Poole. England, UK.

Venous blood sample (10 ml) was drawn from each participant into Vacutainer tubes; containing no anticoagulant, and then centrifuged. The serum samples were separated and stored at -18 ^{o}C. Toenails were obtained by cutting a small piece of nail from the big toe and stored in plastic bag.

Selenium analysis in serum and toenail samples was performed using a Varian AA-880 Zeeman atomic absorption spectrophotometer, coupled to a Vapor Generation Accessory VGA-76 with the ETC-60 Electrothermal Temperature Controller from Varian Techtron Pty. Ltd. Australia to provide hydride generation capability. This system was coupled to SPS3 autosampler and run by SpectraAA worksheet software (Version 4.1). Selenium analysis was performed at a wavelength of 196 nm with 10 mA lamp current. The VGA acid channel must contain 10M hydrochloric acid and the reductant reservoir must contain 0.6% sodium borohydride ($NaBH_4$) in 0.5% sodium hydroxide. The detection limit (DL) for selenium was calculated as 3 x SD in 10 blank solution fortified with 0.2 µg/L selenium was 0.1398 µg/L.

Serum sample of 200 µl was reacted with 1 ml of concentrated "selectipur" nitric acid into a Telfon vessel for two hours. The mixture was heated overnight in the oven at 90^{o}C. When the digestion was complete, and the tubes cooled at room temperature. A 250 µl of 30% hydrogen peroxide was added to each sample. The mixture was heated at 85^{o}C for 30 minutes. The clear digestate was cooled, mixed with 4 ml of 7 M hydrochloric acid and then diluted to 20 ml with deionised water. Before, analysis, samples were heated in water bath at 80^{o}C for 25 minutes. Quadruplicate determinations were made on all samples. Manufacturer's recommendations for wavelength (196 nm), slit width (1.0 nm) and lamp current (10 mA) parameters were followed.

The nails were cleaned with low concentrate detergent to strip off possible contaminants, and then left soaking in a flask with stirring for some time. The samples were then rinsed thoroughly with deionised water and air-dried. Weighed homogenized nail samples of 10 to 50 mg were digested in Teflon vessels with 1 ml concentrated trace metal grade nitric acid for at least five hours at room temperature and then at 95°C for 16 hours. When the digestion was complete, and the tubes cooled at room temperature, 250 µl of 30 % hydrogen peroxide was added and returned to the oven for another 30 minutes. After digestion, the samples were allowed to cool to room temperature. The clear supernatant were transferred to polypropylene tubes and 4 ml of 7 M hydrochloric acid was added and diluted to 20 ml with deionised water. Samples were left in water bath pre-heated to 80°C for 25 minutes and then allowed to cool down to room temperature before analysis.

RESULTS AND DISCUSSION

Calibration selenium standards were prepared each day using a manual standard addition procedure where fresh serum samples (obtained from the Hospital Blood Bank) were divided into six equal portions. Known amounts of aqueous selenium solutions were added to these to give final concentrations in the range of 0.25 to 4.0 µg/L. There was a good linear relation between absorbance and standard concentration of selenium. Linearity was evaluated by calculating the linear correlation coefficients for 29 runs, which was 0.9996 $\pm$ 0.0003. For toenail samples, the calibration curves were prepared each day using a manual standard addition procedure in the range of 0.5 to 8.0 µg/g and the linear correlation coefficients for 23 runs, which was 0.9991 $\pm$ 0.0006. The calibration curve was linear over the specified ranges for serum and toenail selenium as shown in Figure 1.

The accuracy of the analysis of selenium in serum was assessed by analyzing two sets of serum controls with known selenium concentrations: Normal range and High range. The results obtained by analyzing the reference materials were in close agreement with the target concentration values given by the manufacturers. The values found for the Normal and High levels were 117.850 $\pm$ 8.546 and 299.553 $\pm$ 21.945 µg/L respectively, while the expected range for selenium were 87-145 and 245-367 µg/L respectively.

The between-day precision coefficient of variations (CVs) of the Normal and High serum controls were 7.3% and 7.3% respectively. For within-day reproducibility, nine replicates of the Normal and High serum controls were analyzed on the same day. The CVs were 5.7% and 3.7% respectively.

For nail analysis, no certified material was available. Therefore, the accuracy of the method was determined by measuring the recovery of selenium added to pool of homogenized nail samples. These spiked nail samples were run with the test samples using the same analytical procedure. The analytical recovery for twenty spiked nail samples with 0.6, 0.75, 1.5, 3.0 and 6.0 µg/g were respectively 91.200

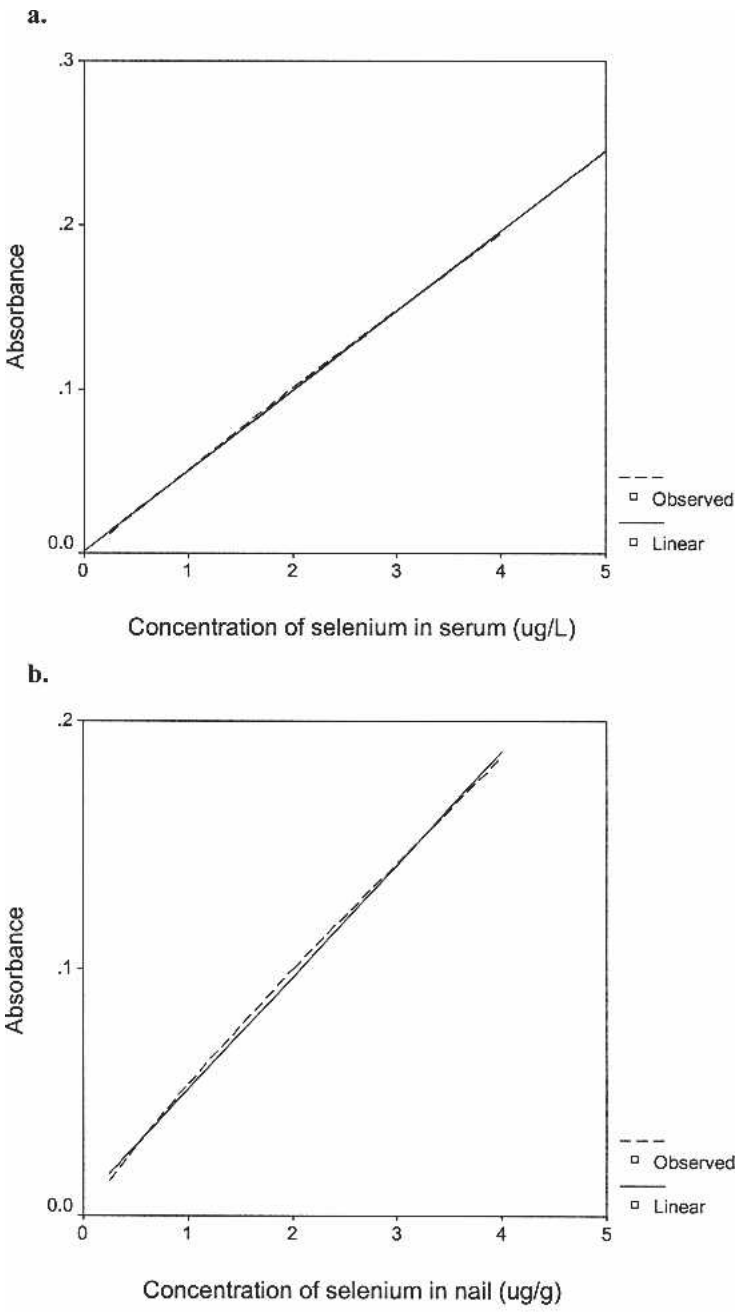

Figure 1. Valid calibration curve for selenium in: (a) serum; and (b) nail samples.

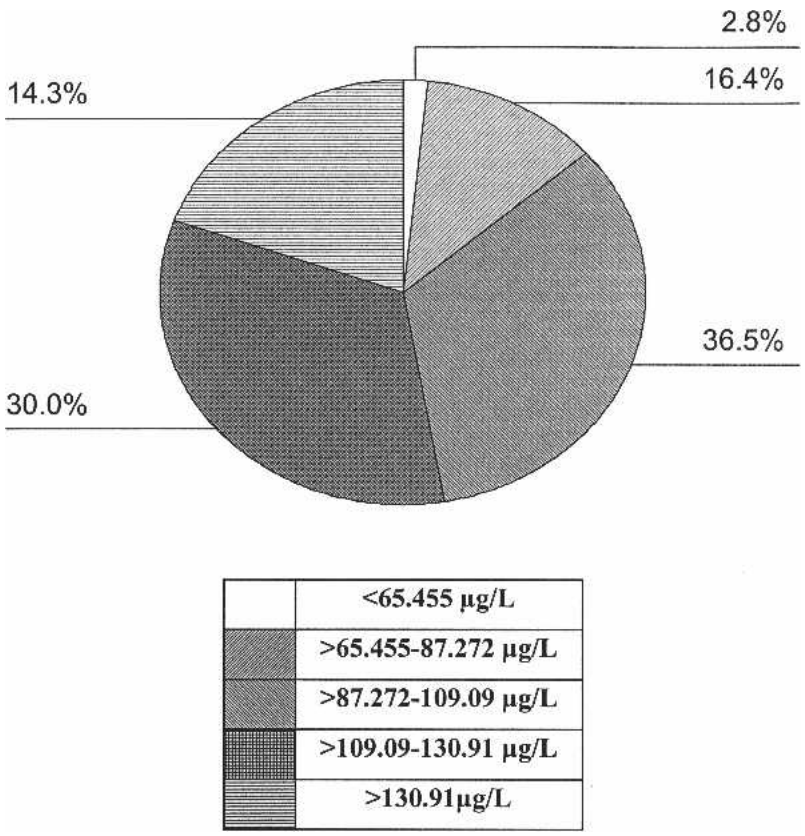

Figure 2. The distribution of serum selenium levels (µg/L) in the studied subjects.

± 9.958%, 94.700 ± 9.462%, 108.075 ± 6.248%, 108.125 ± 8.153% and 103.125 ± 8.475%. The maximum CVs for the within-day and between-day precisions was generally 7.1 and 10.8% respectively.

A total of 743 serum and 691 toenail samples obtained from subjects participating in our cross-sectional study "Selenium and vitamin status in Al-Kharj province and its relationship with endemic diseases" were utilized. The arithmetic mean of selenium in serum and toenail selenium levels were 107.045 ± 23.045 µg/L (n=743, range 52.600-210.120µg/L) and 0.634 ± 0.221 µg/g (n=691, range <DL-1.797 µg/g) respectively. The correlation coefficient between log-transformed values of serum and toenail selenium was -0.158 (p=0). Adjusting for age, gender and BMI decreased the correlation between serum and toenail selenium from 0.158 to -0.163. There might be a number of factors that might affect the levels of

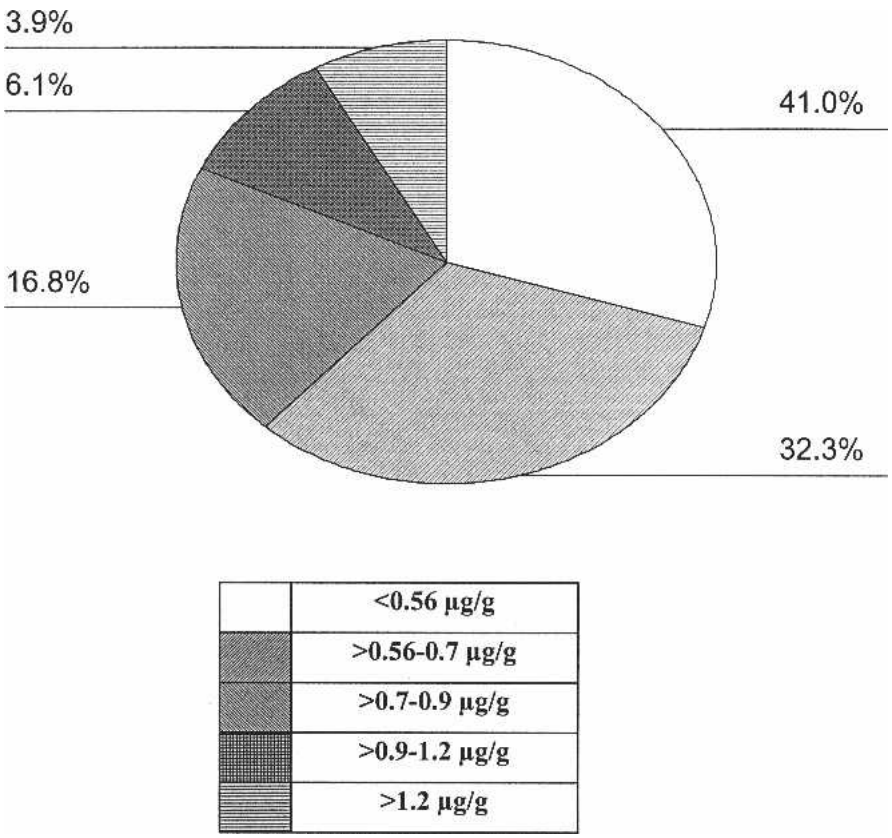

Figure 3. The distribution of toenail selenium levels (µg/g) in the studied subjects.

selenium in toenail compare to serum such as the size of the nail plate in the population to be studied; stresses that slow nail growth; age; and the metabolism of selenium (Longnecker et al. 1993; Hamilton et al. 1955; Hawkes et al. 2003). The chemical form of selenium affects the half-life of selenium in the peripheral tissues and presumably in the nail beds. The half-life of organically bound selenium in peripheral tissues was 15 to 50 day as estimated by Swanson et al (1991). On the other hand, the half-life of selenium from sodium selenite is more than 100 days (Patterson et al. 1989; Hawkes et al 2003). Such differences in metabolism of various selenium compounds may affect the time required to reach steady state in tissues before incorporation in nails. Subjects who are consuming diets with selenite, it would take longer to reach steady state in the toenails compare to selenium in organic form.

Looking at the distribution of serum selenium in the studied population (Figure 2), none of our subjects had serum selenium below the threshold limit of clinical importance in coronary or cardiovascular diseases (45 μg/L), 30% had fallen within the high-above range (100-120 μg/L) of Neve's (1991). However, there were 27.3% of our participants in the range of 120 to 210.120 μg/L.

On the other hand, the distribution of toenail selenium concentrations in Figure 3 revealed that 41% of the tested subjects had toenail selenium <0.56 μg/g, which could be considered low according to Alfthan et al. (1992). Measuring selenium in serum/plasma is the most popular technique for assessing selenium status. But given the wide range of selenium dietary intake which fluctuates according to time as a function of many factors such as the origin of food, a modification in the availability due to environmental causes, or, on the contrary, an enrichment of food with selenium (Varo et al. 1988; Gissel-Nielsen 1986). Neve (1991) stated that plasma selenium might be satisfactory in assessing low to moderate exposure condition since it is quite sensitive to either decrease or increase in selenium. Though, it has a limitation in evaluating increasing selenium status. He attributed this to the differences in selenium homoestastis in relation to the chemical form of selenium. Both serum and toenail selenium levels have been shown to correlate well with selenium intake when measured by using duplicate plate food collection studies (Longnecker et al. 1996; Swanson et al. 1991). However, there is some evidence that toenail selenium may be a more accurate reflection of true long-term average exposure. Toenail selenium has been increasingly used to explore the relationship of prediagnostic selenium intake to cancer risk of breast, colon, prostate and other health conditions such as myocardial infraction (Kok et al. 1989; Ghadirian et al. 2000). Because of toenail selenium content is unaffected by dietary selenium intake during the previous 3 mo but appears to provide a time-integrated measure of exposure over a period ≥26–52 wk (Longnecker et al. 1996). Furthermore, the ease of collecting and storing toenail clippings account for its predominant in assessing exposure in epidemiologic research.

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