

## Role of Selenium and Zinc Deficiency in Etiology of Thyroid Disorder in Khartoum

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### Abstract:

*This study is designed to evaluate the role of trace element (Zinc, Selenium) in the etiology of thyroid disorder in Khartoum area.*

*The overall the prevalence of thyroid disorder in the study group was 41% (140/ 339). The 140 patient were classified in to three different groups according to the serum concentration of TSH.*

*The results of this study suggest that the trace element (Zinc, Selenium) was play and important role in Iodine deficiency.*

**Key words:** road, rockfall, "opened gallery", historical object, environmental protection.

### INTRODUCTION:-

**Selenium:-** Is one of the beneficial elements for animals and human . Since Schwartz and Folts defined Selenium (Se) as an essential trace element, it has been established that Selenium is the functional part of the active center of the 4 types of Se – dependent glutathione peroxidase (GPx). (GPx) is one of the most powerful enzymes of the antiradical and antioxidative defense system of the organism.

Three diseases have been associated with severe Se deficiency: Kashin – Beck disease, Keshan disease, and endemic juvenile cardiomyopathy, and myxoedematous endemic cretinism.

The hypothesis that a relationship existed between Se status and thyroid function was put forward only in mid 1980s. Se is an integral part of the enzyme iodothyronine 5' – deiodinase 1, which converts thyroxine into biologically active triiodothyronine. The human thyroid produces hydrogen peroxide ( $H_2O_2$ ) for oxidative thyroid hormone synthesis. In myxoedematous endemic cretinism, when Se deficiency is combined with Iodine deficiency the oxidative challenge to the thyroid results in postnatally altered thyroid tissue to protect itself from oxidative damage, the cytosolic – GPx catalyzes the reduction of  $H_2O_2$ .

**Zinc:-** Zinc is an essential trace element for humans, animals, plants and microorganisms, the Zinc content in humans is 2 -4 g. Zinc plays many fundamental roles in all replications, gene expression and in the metabolism of nucleic acids and different proteins.

Zinc serves as the metallic portion in over 70 metalloenzymes. Dermatitis, diarrhea, alopecia, mental disturbances, and recurrent infections manifest severe Zinc deficiency, as a result of cell mediated disorders. Chronic Zinc toxicity is characterized by copper deficiency. Acute toxicity is quite rare, as the ingestion of amounts large enough to cause toxicity symptoms (2grams per kilogram body weight) will usually provoke vomiting.

## **SUBJECT, MATERIALS, METHOD:**

### **Subjects:**

Subjects enrolled in the study were newborns, children up to 7 years, young adults 14- 30years, and mothers up to 44 years. The subjects 0-7years were used to study the evolution of

Thyroid status with age. The subjects 14-30 years and the mothers up to 44years were used to determine the involvement of genetic implication in thyroid disorders and to study the age rule from different angle of view. The objective will discussed with mother of newborns and parents of children, and an informed constant will obtained from all subjects included in this study before any enrollment.

Standard questionnaire on food types, educational levels, financial state, history of medical problems and prevalence of disease in the family was filled with information obtained from mother and educated ,Relatives of all the study subjects.

### **Sampling:**

#### **Newborns:**

5cm cord blood samples were collected.

#### **Other subjects:**

5cm venous blood samples were collected from all other subjects.

### **Samples Analysis:**

Sera samples were analyzed for their thyroid hormones [T3, T4, TSH, and Tg] contents. For thyroid hormones and Tg the analysis will performed at the Radioation and Isotopes Center at the Radio Immuno Assay Laboratory {RAL}. Radiometric assay produces were used.

### **Radioimmunometric Assay For TSH, T4 , T3:**

All sera samples used for this analysis were kept clear, and unclear samples were centrifuged first.

**Materials:**

For all assays, PBSX [25mm phosphate buffered saline containing Triton X-100 and Sodium azide] was used. 100ml PBSX were prepared by adding together 5ml stock phosphate, 5ml stock saline 80ml distilled water, 1 ml 10%Triton solution, 0.1g Sodium azide in to a 100ml volumetric flask, and the volume was completed to the mark.

**(i) IRMA Assay buffer:** Prepared by adding PBSX and 10% stock protein solution .90ml PBSX +10ml stock protein solution.

**(ii) Wash buffer:** These were prepared by adding 1ml of 10%Triton solution to 1 liter of distilled water to produced distilled water containing 0.01%TritonX.

**(iii) RIA Assay buffer:** Was prepared by mixing PBSX and 0.1 BSA together in a reagent bottle.

**(iv) RIA tracer buffer** was prepared by mixing PBSX containing 0.9mg/ml ANS and 0.1%ASN and 0.1BSA (50mlPBSX+45MG ASN+50mgBSA).

**(V) Tracer solutions (<sup>125</sup>I-T<sub>4</sub>, <sup>125</sup>I-T<sub>3</sub>, <sup>125</sup>I-TSH):**

5uCiTracer was reconstituted with 1ml distilled water, and stored frozen at 20°C .1uCi<sup>125</sup>I-T<sub>4</sub> , <sup>125</sup>I-T<sub>3</sub>-,<sup>125</sup>I-TSH,were added to each 20ml RIA tracer buffer sufficient for 100 tubes. Counts were checked to give 20.000counts/100sec.

**(vi) Standards:** Each standard was reconstituted with 2ml distilled water, and allowed to stand for 30 minutes with mixing at 10 minute intervals. Aliquots were immediately used and remainder was stored frozen at -20°C.

**(vii) Coated beads:** Prior to opening the bag, coated beads were allowed to warm to ambient temperature.

**Method:**

Test Tubes (75x12mm) sufficient for duplicators standards, quality controls, and samples were numbered with marker pen. The reagents were added as followed: 10ul standard / samples were added followed by 200ul <sup>125</sup>I-tracer solution, vortexes,

followed by the addition of one bead. The whole system was put on a rotator (2 hours in case of T<sub>4</sub>, 1 hour in case of T<sub>3</sub>, and overnight in case of TSH). 2 ml wash buffer were added in to each tube and then all tubes were aspirated. The process was repeated twice. The counter of the analytical instrument was calibrated to perform the analysis within 100 sec.

### **Immunoradiometric Assay For Thyroglobulin in Human Serum :**

**(i) Anti-Tg mAb Coated Tubes:** 2x50 polystyrene tubes (12x75mm) coated with anti-Tg mouse monoclonal antibody.

**(ii) <sup>125</sup>I mAb anti Tg Tracer (red) (2x11ml):** 2 vials <sup>125</sup>I mouse mAb anti -Tg in buffer with stabilizer, a preservative (10-mm Sodium azide) and a red dye. Each vial contains less than 187 KBq (5 μCi) of radioactivity.

**(iii) Tg Standard (1x3ml):** One vial serum medium containing preservative (NaN<sub>3</sub> < 1g/L).

**(iv) Tg Standard S1 to S7 (7x1ml):** 7 vials purified human Tg supplied in serum medium containing preservatives (NaN<sub>3</sub> less than 1g /L). Tg standard curve was calibrated using EUROPEAN CERTIFIED MATERIAL (CRM). The concentration of the different standards were printed on the labels.

**(v) control serum (1ml):** 1 vial human serum containing preservatives. The range of the control serum was printed on the vial label.

**(vi) Washing reagent (100ml):** 1 bottle containing 100 ml of concentrated buffer was provided. The solution was poured in 900 ml of distilled water.

**(vii) Recovery solution (1ml):** 1 vial purified human Tg was supplied in serum medium containing preservatives. The Z value was printed on vial label.

**Method:**

Reagent stored at 2-8°C were brought at room temperature prior to use the tubes were labeled for T (total count - use non coated tubes), standards, samples and control serum. Systematically coated tubes were allowed to reach room temperature before opening the bag. The assay was performed in duplicated. Standards, control and samples were all assayed at the same time.

**(i) Standard Curve:**

100uL each standard solution were added to the corresponding tubes.

**(ii) Test and Control Serum:**

100uL of each sample or control serum was added in to the corresponding tubes, incubated mixture of all tubes was carefully aspirated and decanted. 2ml of washing solution were added to each tube. All tubes were washed for 1 minute, aspirated or decanted carefully. The washing procedure was repeated. 200ul <sup>125</sup>I-mAb tracer (red) were added to each tube. The "Total count" tubes were not participated in the following steps. All tubes were incubated for 2 hours at room temperature on a horizontal shaker. (Max 300rpm). Carefully, the incubation mixture of all tubes (except "total count" tubes) was aspirated or decanted .2ml of washing solution were added to each tube then aspirated or decanted after one minute. The washing procedure was repeated. The radioactivity fixed in each tube was counted for 1 minute in a Gamma scintillation counter.

**Selenium:**

**(A) Materials**

**(i) Stock Standard Solution:**

Selenium 1000mg/L was prepared as follow: 1.000g of selenium metal was dissolved in a minimum volume of concentrated

HNO<sub>3</sub>. The solution was evaporated to dryness. 2ml/sof distilled water were added. Again the solution was evaporated to dryness. The process was repeated 2or 3 additional times .The residue was dissolved in 10% (v/v) HCL and diluted to 1liter with 10%(v/v)HCL.

**(ii) preparation of samples:**

All serum samples were diluted (1+19) with 1%HNO<sub>3</sub>,solution was used as blank solution when determining selenium.

**(iii)Light Sources:**

Both HCL and EDL sources are available for Se, which are more intense, provide better performance and longer life.

**(B)Method:**

**(i) preparation of standard curve:**

Sequence of dilution from the stock solution was made in the ratio 1:3 in order to prepare standards of concentration 33,99 and 198ppm respectively. The diluting principle was based on data obtained from the table of “Standard Atomic Absorption Condition for Selenium”.

**(ii)Analysis:**

The concentration of selenium was determined using the condition listed in the “Standard Condition” table below:

**Standard Atomic Absorption Condition for Selenium**

Wave length(nm)	Slit(nm)	Relative noise	Characteristic Concentration Mg/L	Characteristic Concentration Check Mg/L	Linear Range Mg/L
96.0	2.0	1.0	0.59	30.0	200.0
204.0	0.7	0.61	2.9	150.0	
206.0	0.7	0.44	12.0	600.0	
207.0	0.7	0.43	40.0	2000.0	

1. Recommended flame, air-acetylene, oxidizing (lean, blue).

2. Data obtained with standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer or impact bead will typically provide 2-3x sensitivity improvement.
3. Characteristic Concentration with a  $N_2O-C_2H_2$  flame at 196nm 2.7mg/L.
4. Table contains EDL data. HCL sensitivity values are more than 25% poorer.
5. Se-0.7nm slit on models 3110.

## **Zinc:**

### **(A) Materials:**

#### **(i) Stock Standard Solution:**

Zinc 500mg/L was prepared as follow: 0.500g of zinc metal were dissolved in a minimum volume of (1+1) HCL and diluted to 1 liter with 1% (v/v)HCL.

#### **(ii) Sample preparation:**

For the determination of serum zinc, samples were diluted in 1:5 ratio with distilled water.

#### **(iii)Light Source:**

Both Electrodeless Discharge Lamps (EDLs) and Hollow Cathode Lamps are available for zinc .EDLs provide greater light output and longer life than Hollow Cathode Lamps. For zinc, both EDLs and Hollow Cathode Lamps provide approximately the same sensitivity and detection limit.

### **(B)Method:**

#### **(i)Preparation of Standard Curve:**

Zinc standard were prepared by diluting the stock standard solution for zinc, with 5%(v/v)glycerol.A5%(v/v)glycerol solution was used as blank solution when determining zinc. Standards



from stock solution were prepared in the ratio 1:2:2 such that .025,0.5 and 1mg/L concentration were prepared. The preparation of standards was based principally on data obtained from the table of “Astandard Atomic Condition For Zinc”.

**(ii)Analysis:**

The concentration of zinc was determined using the conditions listed in the “Standard Conditions” table below:

**Standard Atomic Absorption Condition for Zinc**

Wave length (nm)	Slit (nm)	Relative Noise	Characteristic Concentration Mg/L	Characteristic Concentration Check Mg/L	Linear Range Mg/L
213.9	0.7	1.0	0.018	1.0	1.0
307.6	0.7	0.38	79.0	3500.0	

- 1- Recommended Flame: air –acetylene, oxidizing (lean, blue).
- 2- Data obtained with a standard nebulizer and flow spoiler. Operation with a High Sensitivity nebulizer Or impact bead will typically Provide a 2-3 x sensitivity improvement.
- 3- Characteristic Concentration with an N<sub>2</sub>O – C<sub>2</sub>H<sub>2</sub> Flame 213.9 nm 0.084mg/L.
- 4- Table contains HCL data. EDL Sensitivity values approximately the same.

**RESULTS:-**

See Appendix A for Normal Values of all Parameters measured in this study. Thyroid function test was performed on 339 subjects, 0-7 years & 14-30 years from the Khartoum area. The overall Prevalence of hypothyroidism and hyperthyroidism was determined according to values obtained by measuring the level of serum TSH and serum level of the thyroid hormones T3 and

T4. 140 subjects from 339 were found with at least one type of hormonal disorder. The 140 patients were obviously fit into one of 3 different groups. group -1 are those patients with slightly high TSH ( $7.767+2.9169$ ) and normal to slightly lower values of either T3 ( $9756+0.6431$ ) OR  $t4949.54+21.6547$ ) but mainly T4 group2 with higher values of TSH ( $29.88 + 17.9933$ ) and lower values of T4 ( $16.133+13.1196$ ) GROUP -3 lower values of TSH ( $0.1205+0.0858$ ) and higher values of either T3 ( $50293+3.512$ ) OR  $t4$  ( $207.867+49.4612$ ) or he tow hormones together Table distributes the 140 patients into groups according to age criterion.

**Table -1**

	Neonates	<2	2-4	4-7	14-30	Total
N	93	35	59	67	85	339
PATIENTS	21	13	28	37	41	140
%PATIENTS	22.58	37.14	47.45	55.22	48.23	41029

Distribution of 140 patients into classes according to age criterion

Table -2 shows the serum levels of T4, T3 and TSH in the three patients groups.

**Table -2**

Hormones	Group -1	Group -2	Group-3
T4(nmol/l)	$49.54+21.6547$	$16.133+13.1454$	$207.867+50.8191$
T3(nmol/l)	$.9756+0.6431$	$1.053+0.9240$	$5.293+3.3516$
TSH (mu/l)	$7.767+2.9169$	$29.88+17.9933$	$0.1205+0.0813$

Mean  $\pm$ SD serum levels of T4, T3 and TSH in three groups with thyroid disorder.

**Table -3**

(I)TSH	(J)TSH	Mean difference	STD error	Sig
Group1	Group-2	$22.0033^*$	3.843	0.000
Group2	Group-3	6.9313	3.843	0.209
Group3	Group-3	$29.0447^*$	3.843	0.000

Post HOC tests multiple comparison (Scheffe) for three groups of patients , dependent variable TSH.

\*The mean difference is significant at the 0.05 level.

**Table-4**

I	J	Mean Diff(I-J)	STD. Error	Sig.	Lower Boundary	Upper Boundary
Gp1	GP-2	81.2000*	12.808	0.000	48.6964	113.7036
Gp1	GP-3	-110.5333*	12.808	0.000	-143.0369	-78.029
GP2	GP-3	-191.7333*	12.808	0.000	-224.2369	-159.22

Post HOC tests multiple comparison (Scheffe) for three groups of patients, dependent variable T4.

\*The mean difference is significant at the 0.0 level.

79 patients out of 140 (56.42%) were found to be in group -1, 41 patients out of 140 (29.28%) were found to be in group -2 the rest of the patients were in group -3 Table -5 shows the distribution of the 3 group, of patients into classes according to age criterion.

**Table-5**

	Neonates	,2	2-4	4-7	14-30	Total
Group-1	15	6	18	23	17	79
Group-2	6	3	8	11	13	41
Group-3	-	-	3	3	14	20
All group	21	13	28	37	41	140

The distribution of 3 groups into classes according to age criterion.

All patients in all groups were subjected to the Tg test and the results obtained are summarized in table (6).

Table -6 shows the mean & standard deviation of thyroglobulin serum levels of the three groups of patients ,also shows minimum and maximum values obtained for each group.

**Table-6**

	Mean	STD	MIN	MAX
Group-1	140.3797	48.8901	15.00	210.00
Group-2	36.6584	31.3533	12.00	120.00
Group-3	182.0500	47.6726	89.00	250.00
Control	31.4250	16.0318	00.00	65.00

Mean standard deviation minimum and maximum, of thyroglobulin serum levels (ng / ml) of the three groups of patients (140) , and control (n=40).

Tg level is high in all forms of hyperthyroidism, and remains low (normal) in healthy and hypothyroid people <sup>(93)</sup>. In iodine deficient patients with elevated TSH level and normal thyroid hormones level (characteristics of non – toxic goiter), the value of Tg serum levels also rises due to thyroid hyperplasia of iodine deficiency <sup>(94)</sup>. In such cases it reflects iodine nutrition over a period of iodine deficiency <sup>(94)</sup>. In such cases it reflects iodine nutrition over a period of months or years, in contrast to urinary iodine concentration, which assesses more immediate iodine intake.

76 patients (70 from group1 and 6 from group -2 ) were classified as IDD group according to Tg criterion (See table -7) .

**Table-7**

	<b>Group-1</b>	<b>Group-2</b>	<b>Group-3</b>	<b>Control</b>
<b>N</b>	79	41	20	40
<b>LOW Tg</b>	9	35	0	0
<b>High Tg</b>	70	6	20	0
<b>%IDD</b>	88.61	14.6	0	0

\*Percentage prevalence of IDD Patients according to Tg criterion.

All patients possessing both high TG and TSH serum levels were classified as an IDD Group. 76 patients (70 from group-1 and 6 from group-2) fit these criteria. The IDD group was subjected to further tests. Selenium and zinc concentration in the sera of the IDD group patients were measured and the results obtained are summarized in table -8.

**Table -8**

<b>Elements</b>	<b>Zn</b>			<b>Se</b>			<b>Zn &amp; Se</b>			<b>Control</b>		
	<b>L</b>	<b>H</b>	<b>N</b>	<b>L</b>	<b>H</b>	<b>N</b>	<b>L</b>	<b>H</b>	<b>N</b>	<b>L</b>	<b>H</b>	<b>N</b>
<b>Number of individuals</b>	17	-	1	13	-	17	40		6	11	-	40

(Mean±. SD serum level of Zn (mg /l ) and se (umoi/L) in 76 patients and 51 controls).

**Patients:-**

L (Zn)=0.2058+0.0786, N (Zn)=0.7833+ 0.090, L(Se)=0.6311+ 0.0975,

$N(Se)=1.191+0.058.$

**Control:-**

$L(Zn) = 0.3543+0.0550, N(Zn) = 0.8893+0.1416, L(Se) = 0.76 + 0.0826,$

$N(Se) = 1.1548+ 0.0492 .$

Eleven out of fifty one healthy control were found to possess deficiencies of either Zn or Se, but all the 11 were physically and biochemically euthyroid.

The final conclusion is that neither Zn nor Se alone can lead to thyroid disorders , but together are risk factors in iodine deficient subjects increases in the order,  $I<1,Se<I ,Zn<I ,Zn ,Se.$  only 6 patients out of 76 (IDD group) were found to have iodine deficiency accompanied by neither Zn nor Se deficiency . the higher proportion of incidence was found among subjects deficient in all 3 trace elements I, Zn and Se ,followed by individuals deficient in I and Zn , than I and Se . The results of this comparison are shown in table 9&10.

**Table-9**

	Normal	LOW	Total
<b>Patients</b>	19(25%)	57(75%)	100%
<b>Control</b>	44(86.3%)	7(137%)	100%
<b>Total</b>	63	64	

Comparison between an IDD –patients & control, with respect to their frequencies of exhibiting low or normal levels of serum Zn concentrations.

\*person chi – square 45.838 df =1,p< 0001.

**Table (10)**

	Normal	Low	Total
<b>Patients</b>	23	53	76
<b>control</b>	47	4	51

Comparison between an IDD –patients and controls, with respect to their requeencies of exhibiting low or normal levels of serum Se concentrations.

\*person chi-square 47.262,df = 1, p,0.001

## **Conclusion:**

The problem of thyroid disorders in Sudan is not just due to simple environmental factors, other complicated effects are encountered. Trace elements Se, Zn, don't lead to any serious thyroid disorders in iodine sufficient subjects. However, they may increase the risk of the disease in iodine deficient subjects. Thyroglobuline is an important diagnostic tool in most kinds of thyroid disorders. It reflects not only iodine status of subjects but also accounts for some genetic disorders.

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