



## Evaluation of the Iodine Concentration in Serum and Urine of Hypothyroid Males Using an Inexpensive and Rapid Method

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### Abstract

The aim of present study was to evaluate the iodine/iodide status in biological samples (serum and urine) of 172 male hypothyroid patients (HPs) and their supplemental effects on thyroid hormones. For comparison purpose, non-goitrous subjects (n= 220) of same age group and socioeconomic status were also studied. A simple and rapid iodide-ion selective electrode (ISE) was used to measure the concentration of iodine in microwave assisted acid digested serum and urine samples. Quality control for the methodology was established with certified samples and with those obtained by conventional wet acid digestion method on the same certified reference materials (CRMs) and real samples. A linear calibration curve was obtained for a reasonable concentration range of the potassium iodide solutions. The mean concentration of iodine in the serum and urine samples of the HPs was significantly reduced as compared to control male subjects ( $p < 0.01$ ). The low levels of free triiodothyronine and thyroxin were found in HPs than age matched healthy control ( $p < 0.005$  and  $0.002$ ) respectively while high levels of thyroid stimulating hormone were observed in HPs ( $p < 0.008$ ). The proposed method was relatively efficient as well as cost effective by using inexpensive equipment. It was observed that iodine in biological samples of HPs can play an important role in determining the severity of the hypothyroidism.

**Keywords:** Iodine, male, hypothyroid, ion selective electrode, supplements.

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### Introduction

Iodine deficiency could be associated with an increased risk of hypothyroidism due to lack of substrate for thyroid hormones synthesis as observed in area with severe iodine deficiency and it has a central role in thyroid epidemiology [1]. Iodine is an essential nutrient for the normal growth, metabolism and regulation of thyroid hormones in humans and animals [2]. There are large areas in western and central Africa where iodine deficiency disorders (IDDs) are endemic and it is estimated that 250 million people are at risk of IDDs and 50 million have goitrous diseases [3]. Adequate levels of iodine are necessary for proper functioning of

a number of body systems, including the mammary glands, thyroid, salivary glands and the gastric mucosa [4, 5]. Pakistan is a severely iodine deficient country and about 70% of the population was estimated to be at risk for IDD [6]. However, most of the big cities and towns of Pakistan are located within the Indus plain such as Swabi, Peshawar, Lahore, Hyderabad, Dadu, Karachi and Quetta, which have not been extensively investigated [7].

Iodine deficiency influences humans of different age groups such as, men, women (pregnant, lactating, reproductive), and children <3 years [8, 9].

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Antioxidant properties of iodine, which stem from the series of redox reactions underlying iodination of tyrosine leading to formation of thyroid hormones, allow it to reduce the damage, done by major oxidants such as  $H_2O_2$  and other reactive oxidant species [10]. The determination of serum and urinary iodine concentration is a valuable tool in epidemiological studies of iodine supplementation, particularly in diagnosing and controlling iodine deficiency. Because of the difficulty of measuring dietary iodine intake directly, serum and urinary iodine concentrations are usually used as an index of iodine intake [11].

There is great diversity in available serum and urinary iodine analysis methods with respect to cost, technical sophistication, sample processing capacity and performance. Techniques which avoid to destroy potential interfering substances, such as neutron-activation analysis or inductively coupled plasma-mass spectroscopy (ICP-MS) are generally considered methods for iodine analysis but these are unrealistic for widespread use due to high cost and degree of sophistication [12, 13]. On the other hand fewer methods have been described for iodine determination in clinical chemistry [14, 15]. For public health purposes, especially in developing countries, hence there is a need for relatively quicker, simpler and cost-effective methods for determining urinary iodine concentrations.

To the best of our knowledge, no study has been performed to evaluate the status of iodine in biological samples of male hypothyroid patients (HPs) in endemic areas of Pakistan. The present study was undertaken to investigate the concentrations of iodine in biological samples (serum and urine) before and after 6 months treatment with iodine and levothyroxine supplementation. The quantification of iodine was performed by a Potentiometric method (PM), using an iodide-ISE. The other biochemical parameters such as, thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and thyroxine (FT4) were also evaluated. For comparative purpose, same biological samples were also collected from age matched healthy controls subjects residing in same areas (Dadu), Sindh, Pakistan. The serum and urine samples were prepared by the microwave-assisted acid digestion method (MWD), and the validity of the analytical procedure was checked by corresponding conventional wet acid digestion method (CWD) of matrix matched CRMs.

## Material and Methods

### Reagents and glassware

Ultra pure water was prepared by passing de-ionized water from a Milli-Q system (Bedford, USA)

and used throughout the study. All glassware and polyethylene bottles were thoroughly washed with de-ionized water, soaked overnight in 2 M nitric acid and rinsed with ultra pure water before use. Analytical grade chemicals such as nitric acid ( $HNO_3$ )  $\approx$ 16 M, hydrogen peroxide ( $H_2O_2$ ) 30%, potassium iodide (KI) and sodium nitrate were supplied by Merck (Darmstadt, Germany). All the solutions of respective salts and reagents were prepared in ultra pure water. Certified reference material of human serum (SERO-M10181, Billingstad, Norway) and urine NIST SRM 2670a, (Gaithersburg, USA) obtained from the National Institute of Standards & Technology (NIST), were used to check the validation of the proposed method.

### Apparatus

The potentiometric analysis was carried out with an iodide-ISE of Metrohm AG, CH-9101 Herisau (Switzerland) in combination with liquid junction, Ag/AgCl reference electrode using 781pH/ ion meter of Metrohm. A Pel (PMO 23) domestic microwave oven (900 W maximum heating powers) was used for digestion of the samples. A WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge; speeds range 0-6000 rpm was used to separate the supernatant from the sample. Acid washed Polytetrafluoroethylene (PTFE) vessels were used for preparing and storing solutions.

### Study design and pretreatment

An epidemiological study was conducted among male hypothyroid patients ( $n=172$ ) with age ranged (31- 45) years recruited from the outpatient clinic of the nuclear institute of medicine and radiotherapy (NIMRA), Jamshoro, and matched with the same aged non goitrous subjects ( $n=220$ ). Before starting the study, all the normal and thyroid patients were informed by administration through a consent form about the aim of the study and all agreed to participate and signed the form. A questionnaire was also administered to all the normal and hypothyroid patients in order to collect details regarding physical data, ethnic origin, health, dietary habit, age, etc. The clinical examination was carried out, including neck examination by inspection and palpation for goiter according to World Health Organization (WHO) classification [8]. Physical examinations were performed in the basic health unit of NIMRA to measure participant's weight, height, blood pressure and biochemical data. Supplementation, not very different from the RDA levels [16, 17], was given for 6 month to iodine deficient male patients at NIMRA. Hypothyroid patients were supplemented for six month with iodine and levothyroxine (100-125  $\mu$ g/day).

The criteria for the collection of biological samples (serum and urine) of HPs prior to any treatment were such that, the individuals were not taking any mineral supplement during last 3 months. The control male subjects belonged to same socio-economic status and dietary habits, but not suffering from any thyroid problem. The preliminary exclusion criteria for patients and controls were hypertension, alcoholism, smoking, diabetes, cardiovascular disease, taking of any vitamin and minerals.

The venous blood samples (3-5 mL) (including control group) were collected after 12 h fasting, using metal-free safety vacutainer blood collecting tubes (Becton Dickinson, Rutherford ®, USA) between 9.30-11.00 AM. The blood samples were left standing for one hour; sera were separated by centrifugation at 2500 rpm and preserved at -20°C until analysis [18]. For the analysis of other biochemical parameters, up to 5 mL blood samples from same subjects were collected by using metal-free safety vacutainer blood collecting tubes containing >1.5 mg K<sub>2</sub>EDTA and sent to the pathological laboratories of NIMRA. Morning urine samples were collected in an acid-washed, decontaminated 100 mL polyethylene bottles (Kartell1, Milan, Italy). During sampling sessions, each container was wrapped in a clean polyethylene bag. Urine samples were acidified with concentrated HNO<sub>3</sub>, (1 % v/v) and kept at -4 °C. Prior to sub-sampling for analysis, the samples were shaken vigorously for 1 min to ensure a homogeneous suspension.

### **Biochemical assays**

To assess thyroid function, FT4 and FT3 were measured by using radio immuno- assay (Gamma counter, Oakfield, England, SD-12, 2000). Thyroid stimulating hormone was measured by using immuno radiometric assay of the same company. In all patients, measurements of thyroid hormones were performed at first diagnosis time and 6 months after the cure of the disease while in controls they were performed only at study entry. The analysis of serum ferritin (SF), serum albumin (SA) and transferrin receptor (TfR), were also analyzed, as described earlier [19]. Urinary iodine thiocyanate ratio (UI: SCN) was analyzed by a colorimetric method [20] and other biochemical parameters were measured by Medonic CA 620, Haematological Counter (Stockholm, Sweden).

The traditional diagnosis of goiter is based on palpation and World Health Organization (WHO) classification [21], while recent developments in ultrasound technology have facilitated the accurate measurement of thyroid volume (TV). In addition, all subjects were examined by thyroid ultrasonography,

performed with HS-2000 Honda ultrasound equipment with 7.5 MHZ linear probe. The formula we used for calculation of standard TV was based on a model describing the variation in individual TV as a function of several covariates such as age, height and body weight [22].

### **Microwave assisted acid digestion method (MWD)**

A microwave – assisted digestion procedure was carried out, in order to achieve a shorter digestion time. Replicate six certified samples of serum and urine, while duplicate samples of 0.5 mL of serum and urine of each hypothyroid and control individual were directly placed into Teflon PFA flasks. 1.0 mL of a freshly prepared mixture of concentrated HNO<sub>3</sub>- H<sub>2</sub>O<sub>2</sub> (2:1, v/v) were added to each flask and left for 10 min. The flasks were then placed in a covered PTFE container and heated following a one -stage digestion program at 80% of total power (900W), for 2-3 min. After the digestion the flasks containing samples were left to cool, the resulting solution evaporated almost to dryness to remove excess acid, and then diluted to 10.0 mL with 0.1 M nitric acid. The validity and efficiency of the MWD was checked with certified values of CRMs of six replicates biological samples and with those obtained from CWD [23].

Blank extractions (without sample) were carried out by performing the complete procedures without standard and sample. All experiments were conducted at room temperature (30°C) following the well-established laboratory protocols. The resulted digested solutions were analyzed for iodine by iodide-ISE.

### **Potentiometric method**

Potentiometric analysis for the determination of serum and urinary iodine was carried out by iodide-ISE [24, 25]. A linear range of iodine standards was prepared from 12.7 to 127000 µg/L (0.1 µM to 1mM) KI, at 30 °C and membrane resistance (MΩ) < 0.1. The calibration was found linear in the entire range of iodine concentration with a slope value of -59.8 mV. The equation for the linear range of the iodine calibration curves was the following:  $X = -59.8(I) - 47.073$ ,  $R^2 = 0.9998$ .

Where X is integrated electromotive force (EMF) expressed in millivolts (mV) and the concentration in each case expressed as µg/L. The limit of detection (LOD) and limit of quantification (LOQ) for iodine/iodide were calculated by using equation,  $LOD = 3 \times s/m$  and  $LOQ = 10 \times s/m$  respectively, where “s” is the standard deviation of ten measurements of the

blank and “m” is the slope of the calibration graph. The LOD and LOQ of iodine were 1.96 and 6.52  $\mu\text{g/L}$  respectively. This showed that the iodide-ISE was very much sensitive for the determination of serum and urinary iodine. The sample analysis were carried out by taking 1 mL each of serum and urine samples in 10 mL volumetric flasks, added 0.5 mL of 2 M  $\text{NaNO}_3$  as total ionic strength adjusting buffer (TISAB) and diluted up to the mark. The iodide-ISE was immersed into the sample, the solution stirred gently for a few seconds and after 3 min iodine was measured in  $\mu\text{g/L}$ .

The validity and efficiency of the MWD was checked by the values, obtained from CWD [25]. The indicative values for both protocols were calculated as the arithmetic means of 6 replicate of human serum (SERO-M10181, Billingstad, Norway), and human urine NIST SRM 2670a, (Gaithersburg, USA) were used, as shown in Table 1. The MWD was efficient and took less than 5 min to complete the digestion of the certified biological samples. The overall recoveries of iodine in certified biological samples, by using the MWD as compared to CWD were 98.5% - 98.8% in CRMs of serum and urine respectively. Mean values of iodine varied less than 1-2 % from the certified values. The coefficient of variation changed < 2%.

**Table 1. Determination of Iodine in Certified Samples by Conventional Wet Acid (CWD) and Microwave Digestion Method (MWD) (N=6).**

Iodine <sup>b</sup>	Certified values	CWD	MWD	T value <sup>a</sup>	% recovery
					98.8
Serum	93.6±1.74	93.3±1.88 (2.02)	92.2±1.77 (1.92)	0.020	
Urine	88.2±1.24	88.8±1.94 (2.18)	87.5±1.80 (2.06)	0.015	98.5

**Key:** <sup>a</sup>Paired t-test between CWD and MWD DF=5,

T (critical) at 95% CI = 2.57

Values in ( ) are RSD

<sup>b</sup>Iodine concentrations measured as for iodide in  $\mu\text{g/L}$

<sup>c</sup> % recovery was calculated according to:  $\frac{[\text{MWD}]}{[\text{CWD}]} \times 100$

The accuracy of analytical method was performed with certified samples and triplicate analysis of serum and urine samples spiking with known amount of iodine in biological samples. Their values and percentage recovery are given in Table 2.

The precision was calculated as the coefficient of variation (C.V. %) within a single run (intra-assay) and between different runs (inter-assays). Pooled serum and urine samples with low, medium, and high concentrations of iodine were used to determine the intra- and interassay CVs. At medium and high

concentrations of serum and urinary iodine, the intraassay CVs were < 5.2 %, and the Interassay CVs were < 5.3 % Table 3.

**Table 2. The Results for Tests of Recovery for Serum and Urinary Iodine (N=3).**

Added ( $\mu\text{g/L}$ )	$\bar{x}$ CWD	Iodine found ( $\mu\text{g/L}$ )		
		Recovery (%)	$\bar{x}$ MWD	Recovery (%)
0	43.3 ± 0.64	-	42.4 ± 0.42	-
5	48.2 ± 0.52	98.8	47.3 ± 0.38	98.2
10	53.2 ± 0.55	99.3	52.2 ± 0.35	98.2
15	58.3 ± 0.45	99.7	57.2 ± 0.42	98.8
20	63.3 ± 0.52	98.8	62.2 ± 0.78	98.2

Average values ± standard deviation (N= 3).

**Table 3. Intra-assay coefficient of variation (CV) and interassay CV of the tests used to measure iodine in the serum and urine samples ( $\mu\text{g/L}$ ).**

Serum	Sample	Mean ± SD	CV %
Intraassay (10 replicates)	Low	22.9 ± 1.62	7.1
	Medium	35.1 ± 1.84	5.2
	High	52.6 ± 2.05	3.9
	Low	20.2 ± 1.58	7.8
	Medium	34.4 ± 1.82	5.3
	High	50.1 ± 2.03	4.1
Urine	Low	40.2 ± 2.25	5.6
	Medium	63.8 ± 2.32	3.6
	High	112 ± 2.63	2.4
	Low	38.2 ± 2.22	5.8
	Medium	62.9 ± 2.3	3.7
	High	106 ± 2.62	2.5

### Statistical analysis

Data processing and statistical analysis were conducted by using computer program EXCEL (XP

2002; Microsoft Corp., Redmond, WA) and Minitab 13.2 Minitab Inc., State (College, PA) software packages. Data were expressed as means  $\pm$  std and Student's t-test was used to assess the significance of the differences between the variables investigated in hypothyroid and control male subjects.

## Results and Discussion

The present study investigated the possible differences in iodine concentration among biological samples and thyroid hormones levels in male hypothyroid patients and the effect of iodine and levothyroxine supplementation on measures of iodine and thyroid hormone metabolism.

The results of the biochemical parameters in HPs before and after treatment are shown in Table 4. There were no significant differences in weight and body mass index of male hypothyroid and normal subjects. The HPs has lower hemoglobin (Hb) % as compared to recommended range for normal persons (11.5–16.5 g/dL). The SF, Haematocrit (Hct) and UI:SCN levels before treatment were significantly lower in HPs than normal subjects ( $p < 0.007$ ,  $0.005$  and  $0.01$  respectively). The levels of TfR and TV in HPs were significantly higher than control subjects ( $p < 0.05$ ). The other blood parameters such as, SA, erythrocyte or red blood cells (RBC) and white blood cells (WBC) show no difference in hypothyroid and control male subjects ( $p > 0.05$ ). After the six months treatment with iodine and levothyroxine supplementation, all the biochemical parameters were maintained to normal level but still lower than control subjects Table 4.

The levels of serum and urinary iodine in HPs before treatment were significantly lower as compared to controls subjects ( $p < 0.01$ ). After six months treatment with iodine and levothyroxine to HPs, the concentration of iodine in serum and urine was increased in the range of 44.5–60.6 and 58.9–86.2  $\mu\text{g/L}$ , respectively, as given in Table 5.

The concentrations of TSH in HPs before treatment was found at 95% confidence interval [CI: 4.72, 6.22]  $\mu\text{IU/mL}$ , was significantly higher than control subjects ( $p < 0.008$ ), while after treatment, the TSH levels in HPs was decreased [CI: 4.12, 5.45]  $\mu\text{IU/mL}$  (Table 6). The concentrations of FT3 and FT4 before treatment were found to be in the range of 1.65–2.46 and 8.7–10.6 pmol/L in HPs significantly lower as compared to controls ( $p < 0.005$ ) respectively. However, after 6 month of treatment, FT3 and FT4 levels in HPs were increased as shown in Table 6.

The correlation of serum and urinary iodine levels vs. TSH, FT3 and FT4 in hypothyroid male patients was statistically analyzed by multiple linear regression equation and Pearson correlation Table 7.

**Table 4. Biochemical Parameters of Normal and Male Hypothyroid Patients (HPs) before (PBT) and after Treatment (PAT).**

Parameters	Normal	PBT	PAT	Normal Range
Weight (kg)	64.7 $\pm$ 6.4	60.2 $\pm$ 5.5	63.8 $\pm$ 5.8	.....
Height (cm)	156 $\pm$ 5.5	154 $\pm$ 5.2	154 $\pm$ 5.2	.....
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 3.7	25.4 $\pm$ 3.5	26.9 $\pm$ 3.9	.....
Hb (g/dL)	12.8 $\pm$ 3.52	9.1 $\pm$ 3.3	11.4 $\pm$ 3.4	11.5-16.5
SF ( $\mu\text{g/L}$ )	42.5 $\pm$ 4.8	22.4 $\pm$ 4.3	30.8 $\pm$ 4.6	>30
SA (g/dL)	4.4 $\pm$ 0.75	3.42 $\pm$ 0.70	5.1 $\pm$ 0.85	3.4-5.4
Hct (%)	46.2 $\pm$ 2.8	32.8 $\pm$ 2.4	38.3 $\pm$ 2.6	35-55
TfR (mg/L)	8.1 $\pm$ 2.5	25.8 $\pm$ 5.4	16.5 $\pm$ 4.5	2.9-8.5
TV (mL)	14.6 $\pm$ 3.5	27.4 $\pm$ 5.5	17.8 $\pm$ 4.6	< 25
UI:SCN ( $\mu\text{g/mg}$ )	4.3 $\pm$ 0.92	1.9 $\pm$ 0.56	2.8 $\pm$ 0.72	>3
RBC (mm <sup>3</sup> )	4.6 $\pm$ 0.69	3.45 $\pm$ 0.42	4.4 $\pm$ 0.62	3.5-5.5
WBC (mm <sup>3</sup> )	8.7 $\pm$ 0.82	6.42 $\pm$ 0.5	7.6 $\pm$ 0.7	3.5-10
MCV ( $\mu\text{m}$ )	91.5 $\pm$ 6.2	65.7 $\pm$ 4.8	81.9 $\pm$ 5.8	75-100
MCH (pg)	32.6 $\pm$ 2.6	22.3 $\pm$ 2.1	29.1 $\pm$ 2.4	25-35

**Key:** BMI: body mass index, Hb: hemoglobin, SF: serum ferritin, SA: serum albumin, Hct: haematocrit, TfR: transferrin receptor, TV: thyroid volume, UI:SCN: urinary iodine thiocyanate ratio, RBC: red blood cells, WBC: white blood cells, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin. Mean  $\pm$  standard deviations were calculated for all biochemical parameters.

**Table 5. Iodine Concentrations in Serum and Urine Samples of Normal and Male Hypothyroid Patients (HPs) before (PBT) and after Treatment (PAT) ( $\mu\text{g/L}$ ).**

Male	Normal	PBT	PAT
Serum	83.6 $\pm$ 11.4	44.5 $\pm$ 11.5	60.6 $\pm$ 11.7
Urine	123 $\pm$ 15.8	58.9 $\pm$ 12.3	86.2 $\pm$ 13.2

**Table 6. Hormonal Status in Normal and Male Hypothyroid Patients (HPs) before (PBT) and after Treatment (PAT).**

Male	Normal	PBT	PAT
TSH ( $\mu\text{IU/mL}$ )	3.95 $\pm$ 1.42, [3.14, 4.72]	5.61 $\pm$ 1.9, [4.72, 6.22]	5.12 $\pm$ 1.72, [4.12, 5.45]
FT3 ( $\text{pmol/L}$ )	4.2 $\pm$ 0.66, [3.52, 4.63]	2.3 $\pm$ 1.1, [1.65, 2.46]	3.62 $\pm$ 1.2, [2.9, 4.14]
FT4 ( $\text{pmol/L}$ )	16.8 $\pm$ 2.82, [14.9, 18.34]	8.92 $\pm$ 2.35, [8.7, 10.6]	12.2 $\pm$ 2.5, [10.33, 14.5]

Key: Values in [ ] At 95.0% confidence limit

**Table 7. Linear Regression and Pearson Coefficient of (Serum and Urinary iodine Vs. TSH, FT3 & FT4) in Male Hypothyroid Patients (HPs).**

Male	TSH	FT3	FT4
Serum iodine	r=0.66, (0.007)	r=0.55, (0.002)	r=0.72, (0.006)
Urinary iodine	r=0.58, (0.006)	r=0.52, (0.002)	r=0.70, (0.005)

Key: Values in ( ) are p-value

### Methodology

The epidemiological study provided data of iodine concentrations in serum and urine as well as level of thyroid hormones (TSH, FT3 and FT4) obtained from the male hypothyroid patients and control subjects of developing country, Pakistan. The results of previous studies revealed that iodine deficiency still continues to be prevalent in Pakistan [25, 26].

Iodine loading test is widely used and the measurement of serum and urinary iodine levels are best performed in medical practice, using a simple procedure with non-hazardous reagents. The proposed method is accurate, precise and cost effective because it lacks the use of suitable and comparatively expensive chemicals as needed in colorimetric method for determining serum and urinary iodine [25]. Moreover, the instruments used in colorimetric method are expensive as compared to the use of ion meter in the case of iodide-ISE. The reagents used in this procedure are non-toxic and well suited for frequent determination in clinical study. Sodium nitrate ( $\text{NaNO}_3$ ) was used as an isotonic strength adjuster (ISA) at an initial concentration of 5 M to improve performance of the electrode. The ISA was diluted by one part of ISA and two parts of iodine standards and samples and the final concentration of  $\text{NaNO}_3$  in the assay was 2 M. The average cost by our proposed method is about 40 rupees/sample, while the

colorimetric method has an average cost of >200 rupees/sample. The proposed method can be applied at commercial scale due to its rapidity and economy as compared to other reported method for iodine determination and it is also a substitute for routine biochemical tests for hypothyroidism.

Validation for interference is an important part of iodine determination, particularly for those using milder forms of digestion to remove physiologically important interfering substances from serum and urine [27, 28]. No digestion method can be guaranteed to completely remove all potentially interfering substances, many of which occur only in trace amounts. The treated MWD improves the determination of iodine in serum and urine as compared to CWD digestion. The average time taken in microwave determination for single sample is about half an hour by iodide-ISE, while other tests are more time consuming completed in more than one hour, due to use of conventional digestion and further preparation of sample for analysis.

Several different methods for the detection of iodine have already been developed. One relatively early method known as the Sandell and Kolthoff reaction involves a colorimetric assay of the redox reaction between Ce (IV) and As (III), which is catalyzed by iodine [29]. The rate of disappearance of the characteristic yellow color of Ce (IV) can be used to estimate the concentration of iodine in samples. There are problems when various substances or contaminants are present in the sample that would reduce Ce (IV) and result in unacceptably high margins of error.

### Sensitivity

The sensitivity of the iodide-ISE electrodes for iodine measurement is  $5 \times 10^{-8}$  M. The iodide-ISE is by far the most sensitive, being 20, 100 and 1000 times more sensitive than fluoride, bromide and chloride ISE respectively [24].

### Specificity of the Ion-selective electrode and possible interferences by other ions

According to information supplied by Metrohm, chloride will interfere with the iodide-ISE if the chloride/iodide molar ratio exceeds to 1 million. Although the iodide-ISE is very specific for iodine, chloride may interfere in the assay of iodine when the molar concentration of chloride in the urine is six orders of magnitude greater than that of iodine [30]. For bromide iodide-ISE is less specific, with expected interference when the bromide/iodide molar ratio exceeds to 5,000 [30]. As the average concentration of chloride and bromide in serum samples is estimated as

101 mM [31] and 4.13 mg/L [32] and in urine as 126.2  $\mu$ M [31] and 0.3 – 7.0 mg/L [33], which is quite lower than iodide concentration as reported [30], hence no interference is expected from the mentioned ions during iodide determination by ISE.

#### ***Validity of MWD method***

It is clear in table 2 that the concentration of iodine obtained by MWD and CWD are closer and within acceptable range of standard deviation. This confirms that MWD is highly valid for the mentioned assay in urine as well as serum samples. The method has the advantage over CWD for economizing the time of analysis meaning thereby that one can analyze greater number of samples in lesser time.

#### ***Iodine deficiency in male hypothyroid patients***

Hypothyroidism is a clinical entity resulting from deficiency of iodine and thyroid hormones and in that case basal metabolic rate is decreased [34]. Poor nutrition is considered one of the causes of an under active thyroid; thus, providing optimal nutrition is vital to correcting them, as well as to prevent further decline.

Healthy thyroid function is dependent on a range of nutrients, especially iodine, iron, zinc, copper, selenium and tyrosine [16, 23, 25]. In addition to proper nutrition, fluctuations in hormone levels may also act as a trigger in thyroid dysfunction, resulting in a sub-clinical thyroid imbalance in the body of HPs. It was reported that, the phytic acid in human inhibit absorption of iodine, iron, zinc, calcium, magnesium and manganese [35]. Maintained adequacy of iodine intake in populations is dependent on consuming food with sufficient natural iodine content or food supplemented with iodine. Dietary iodine is ingested in a variety of forms and most of it is reduced in the gut to iodide and rapidly absorbed [36]. Most of the iodide is transported into the thyroid and incorporated into thyroid hormones. Generally, iodine status is characterized from serum and urinary iodine excretion. This is plausible; approximately 90% of ingested iodine is excreted in the urine whereas the remaining 10% is excreted in faeces, however with large inter-individual differences [37].

In adults and adolescents who consume adequate amount of iodine, most dietary iodine eventually appears in the urine; thus, the urinary iodine concentration is currently the most practical biochemical marker for determining the iodine nutrition of populations at the time of measurement [8]. Akhter et al. [38] attempted to estimate the daily dietary intake of iodine by Pakistanis, reported high prevalence rates of

IDDs and supported the use of iodized salt as a corrective strategy. The results of our study substantiate growing concerns that dietary iodine deficiency is increasing in understudy area. We need to know whether the recent influx of studies demonstrating iodine deficiency, indicates a re-emergence, or an ongoing problem, of iodine deficiency in adults. The accumulating facts for iodine deficiency in subjects of our study suggested that iodine intake might be lower.

The fact shows that serum and urinary iodine levels were low in hypothyroidism and high in hyperthyroidism [39]. The present study showed a lower serum and urinary iodine excretion in HPs than control subjects ( $p < 0.01$ ), and a significant correlation has been found between serum and urinary level of iodine and TSH, FT3 and FT4 levels Table 5 & 7. Brauer et al. [40] has reported the low level of urinary iodine excretion than normal in hypothyroid patients. The findings of our study are parallel to the above-mentioned reports which claim that iodine deficiency has a negative effect on thyroid hormone functions.

The measurement of the biochemical parameters such as, Hb, SA, SF and WBC appears to be more useful indicator of iodine status as shown in Table 4. Low intake of dietary iodine was probably the primary dietary factor responsible for the low biochemical status of these hypothyroid patients [41]. Thyroid ultrasonography is a precise and objective method for measuring goiter size that has become feasible for field studies even in remote areas and it is particularly valuable for accurate detection of small goiters in adult men [42]. The reports of understudy patients indicated that a TV for male was higher than normal level as shown in Table 4.

#### ***Effect of supplementation***

In the developing countries like Pakistan, due to high rate of poverty and illiteracy in most of population, the amount of iodine absorbed from diet is not sufficient to meet many individual's body requirements. The main sources of iodine are animal based food: fish, meat, etc and it is also naturally high in iodized table salt and iodine-rich foods (seafood and green, leafy vegetables). The consumption of predominantly cereal based diet, rich in phytate, oxalate, phosphate, fiber and other inhibitor of iodine absorption which decreases iodine bioavailability was the main causes of iodine deficiency in humans [43]. Recommendations by the International Council for the Control of Iodine Deficiency Disorders, WHO, and UNICEF [8] set the minimal urinary iodine concentration for iodine sufficiency as 100  $\mu$ g/L; this figure corresponds roughly to a daily intake of 150

iodine  $\mu\text{g/day}$ . There was also a trend toward decreases in thyroid hormone concentration during the low iodine concentration. The significant decrease in FT3 and FT4 should have been accompanied by a reciprocal increase in TSH as part of the normal feedback control of thyroid hormones [44].

The supplementation is effective treatment of hypothyroidism necessary to meet requirements for growth, and replenishment of depleted body stores. Supplementation of iodine and levothyroxine (100-125  $\mu\text{g/day}$ ) were given for 6 month to HPs. The levothyroxine sodium dose was generally adjusted in 12.5-25  $\mu\text{g}$  increments until the patient with hypothyroidism is clinically euthyroid or normal and the level of TSH has been normalized. It was reported in literature that iodine and levothyroxine supplementation was associated with a lower incidence of hypothyroidism and thyroid inflammation. [45]. In our study, HPs consuming diets which had poor iodine status. The supplementation period as recommended by consultant was six months, resulted in increased concentration of Hb, SA, SF, UI: SCN, RBC and WBC, while decreased the contents of TfR and TV as shown in Table 4.

In our study, iodine and levothyroxine supplementation resulted in significant increases in serum and urinary iodine concentrations ( $p < 0.001$ ) in HPs indicating that iodine status was improved Table 5. It was also observed that thyroid hormone levels in hypothyroid patients fed with iodine and levothyroxine supplement were increased but still lower than controls as shown in Table 6. Importance of our study finding was that the supplementation enhance the levels of serum FT3 and FT4 in both gender, while decrease the TSH levels, these results are consistent with other study [46].

### Conclusion

Potentiometric analysis of serum and urinary iodine by iodide-ion selective electrode is an ideal method for a clinical testing, requiring only water and sodium nitrate as reagents. The microwave assisted acid digestion made our method more rapid. We further suggest that ion selective electrode procedure is simpler and economical as compared to other methods in order to encourage physicians and other healthcare providers to perform the loading test on thyroid patients to measure serum and urinary iodine levels. Using the ISE method, it was observed that after six month treatment with iodine and levothyroxine supplementation in male hypothyroid patients have still lower values of urinary iodine as compared to control subjects.

It was also concluded that iodine deficiency is a major risk factor in Pakistan and iodization programme seems justified, but due to possible side effects, monitoring is mandatory and the optimal intake of iodine still need to be established. A wide-scale epidemiological study is recommended, together with the examination of the potential preventive role of iodine supplementation in endemic goiter areas.

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