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DOI: 10.46903/gjms/19.04.935

ORIGINAL ARTICLE

SERUM LEVELS OF OXIDATIVE STRESS MARKERS IN SUBCLINICAL AND OVERT HYPOTHYROIDISM VERSUS CONTROL GROUP IN POPULATION OF KUTAHYA CITY, TURKEY

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ABSTRACT

Background: Overproduction of oxygen-related free radicals and inadequate antioxidant defense are critical in hypothyroidism, as they might lead to future life-threatening diseases. The objectives of this study were to compare serum levels of oxidative stress markers; total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), paraoxonase-1 (PON-1), and arylesterase (ARYL) in patients with subclinical hypothyroidism (SH) and overt hypothyroidism (OH) versus healthy controls in population of Kutahya city, Turkey.

Materials & Methods: This cross-sectional study was conducted at Department of Biochemistry, Faculty of Medicine, Kutahya University of Health Sciences, Kutahya, Turkey, from July to December, 2016. Three study groups of 45 each were formed as healthy controls, patients with SH and OH. Serum levels of TOS, TAS, OSI, PON-1, and ARYL were five research variables with ratio scale, but skewed, so described by median and IQR with 95% CI. Kruskal-Wallis and multiple comparison tests were used for hypotheses testing.

Results: Serum levels of TOS and OSI were higher in SH group (p-values 0.032; <.0001; respectively) and OH group (p-values <.0001; <.0001, respectively) than healthy controls, while serum levels of TAS, PON-1 and ARYL were lower in SH group (p-values <.0001; <.0001; <.0001, respectively) and OH group (p-values <.0001; <.0001; <.0001, respectively) than control group.

Conclusion: Our study showed that serum levels of TOS and OSI were higher in subclinical and overt hypothyroidism than in healthy controls; while serum levels of TAS, PON-1, and ARYL were lower in subclinical and overt hypothyroidism than in healthy controls.

KEY WORDS: Hypothyroidism; Thyroid Stimulating Hormone; Oxidants; Antioxidants; Oxidative Stress; Paraoxonase-1; Total Oxidant Status; Total Antioxidant Status; Oxidative Stress Index; Arylesterase.

Cite as: Yontem M, Arslan S, Erdogdu BS, Kocak FE. Serum levels of oxidative stress markers in subclinical and overt hypothyroidism versus control group in population of Kutahya city, Turkey. Gomal J Med Sci 2021 Oct-Dec; 19(4):132-40. https://doi.org/10.46903/gjms/19.04.935

1. INTRODUCTION

1.1 Background: Hypothyroidism is a common metabolic disorder characterized by a slowed metabolism caused by low thyroid hormone levels in the blood. Overt hypothyroidism (OH) is defined by lower serum thyroid hormone levels and higher thyroid stimulating hormone (TSH) levels than the normal ranges. Subclinical hypothyroidism (SH) occurs when TSH levels are elevated, while serum thyroid hormone levels are within the normal range.[1]

SH is a growing global public health problem that affects 4-20% of the adult population and has an even higher incidence than diabetes.[2] Many metabolic parameters are regulated by thyroid hormones, thus the alterations in the secretion of TSH and thyroid hormones affects various systems, including the musculoskeletal, respiratory, gastrointestinal, nervous and cardiovascular systems.

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Date Submitted: 06-08-2020
Date Revised: 13-07-2021
Date Accepted: 17-08-2021

The imbalance between the oxidant and antioxidant defense mechanisms is known as oxidative stress and the oxidative stress index (OSI) is calculated by dividing total oxidant status (TOS) (μmol H2O2 equivalent/L) to total antioxidant status (TAS) (mmol Trolox equivalent/L).[7] Overproduction of reactive oxygen species or failure of various antioxidant defense systems cause molecular dysfunction by oxidizing macromolecules like carbohydrates, lipids, and proteins.[8] Due to slowing down effects on the metabolism, free radical production is expected at low rates in OH.[9,10] Contrary to this, some researchers have revealed that oxidative stress is elevated in OH.[11-13] It seems that the values of the OSI in OH and SH are important in providing information about the status of antioxidant defense in hypothyroidism.

Paraoxonases (PON) are an enzyme family, consisting of three members; PON-1, PON-2, and PON-3. Both PON-1 and PON-3 have antioxidant and anti-inflammatory properties.[14] PON-1 has a protective role by hydrolysing lipid peroxides of oxidized lipoproteins.[15-17] In addition, as a result of increased oxidative stress, reduced PON-1 activities are reported in many disorders such as diabetes, hypercholesterolemia, and cardiovascular disease.[18-20] Moreover, PON-1 plays an important role in enzymatic antioxidant defense with arylesterase (ARYL) which has a similar function with PON-1.[21]

1.2 Research Objectives (ROs)

RO 1-5: To analyze the serum levels of oxidative stress markers; TOS, TAS, OSI, PON-1 & ARYL in control, SH, and OH groups

RO 6-10: To compare the serum levels of oxidative stress markers; TOS, TAS, OSI, PON-1 & ARYL in SH and OH versus control group.

1.3 Research (Null) Hypotheses (RHs)

H_{01}: Serum level of TOS is same in SH as control group in population of Kutahya city, Turkey.

H_{02}: Serum level of TOS is same in OH as control group in population of Kutahya city, Turkey.

H_{03}: Serum level of TAS is same in SH and control group in population of Kutahya city, Turkey.

H_{04}: Serum level of TAS is same in OH as control group in population of Kutahya city, Turkey.

H_{05}: Serum level of OSI is same in SH and control group in population of Kutahya city, Turkey.

H_{06}: Serum level of OSI is same in OH as control group in population of Kutahya city, Turkey.

H_{07}: Serum level of PON-1 is same in SH as control group in population of Kutahya city, Turkey.

H_{08}: Serum level of PON-1 is same in OH as control group in population of Kutahya city, Turkey.

H_{09}: Serum level of ARYL is same in SH as control group in population of Kutahya city, Turkey.

H_{010}: Serum level of ARYL is same in OH as control group in population of Kutahya city, Turkey.

2. MATERIALS & METHODS

2.1 Design, Duration & Setting: From July to December 2016, this sectional study was conducted in the Department of Biochemistry, Faculty of Medicine, Kutahya University of Health Sciences, Kutahya, Turkey. The samples were selected from the Department of Medical Biochemistry, Evliya Celebi Research and Education Hospital, Kutahya, Turkey. The Declaration of Helsinlevelski's guidelines were followed. Ethical committee approval was granted by the local Human Research Ethics Committee. Written informed consent was obtained from all subjects.

2.2 Population & sampling: Kutahya city is in the western part of Turkey with assumed population of 450,000 for the year 2016. Three samples each of 50 adult subjects were selected from this population as group 1 healthy control, group 2 SH and group 3 OH cases.

Clinically and laboratory confirmed new cases of SH and OH were included. Exclusion criteria were as follows: thyroid hormone therapy in past, alcoholism, smoking, existence of chronic medical illness, chronic pharmacological therapy, usage of supplemental multivitamins, minerals and antioxidants.

SH was diagnosed by high serum TSH level (>5.6 mIU/mL) associated with normal fT4 level. OH was diagnosed by high TSH level associated with low fT4 level (<0.34 mIU/mL).

2.3 Collection of blood samples and analysis of laboratory parameters

2.3.1 Blood sample collection

Biochemical studies were performed on venous blood samples. Blood samples were centrifuged at x 1500 g for 15 minutes. Serum samples were stored at -80°C for TAS, TOS, PON-1, and ARYL assays.

2.3.2 Measurement of serum TAS and TOS levels

Serum TAS and TOS levels were measured using Erel's new automated analysis technologies.[22,23] TAS levels were expressed in millimoles of Trolox equivalent per liter. TOS levels were expressed in μmol H_2O_2 equivalent per liter.

2.3.3 Calculation of OSI

The OSI was established as a measure of oxidative stress based on the percent ratio of TOS to TAS. The OSI was calculated as follows: OSI= [(TOS, μmol H_2O_2 Eq/L) / (TAS, μmol Trolox Eq/L) x 100].[24] OSI values were expressed as arbitrary units (AU).

2.3.4 Measurement of serum PON-1 and ARYL activities

PON-1 and ARYL activity in the serum were analyzed using Erel's automated measurement methods.[25,26] The activity of PON-1 and ARYL in the serum were expressed in units per liter (U/L).

2.3.5 Measurement of serum fT4 and TSH levels

The chemiluminescent immunoassay method was used to quantify serum fT4 and TSH levels. TSH: 0.34-5.6 mIU/mL, fT4: 7.85-14.41 pmol/L, were the reference values in healthy individuals.

2.4 Data collection & data analysis plan

2.4.1. Descriptive statistics & estimation of parameters

Five research variables were serum TOS, TAS, and OSI levels, as well as PON-1 and ARYL activity. The data type for these variables was ratio (numeric). The skewness, kurtosis, coefficient of variation percent (CV percent), and Shapiro-Wilk (SW) tests were used to determine normality. SW test statistics (W) and p-values were presented. As all data was skewed, so it was analyzed by median (Q2), quartile 1 (Q1), Q3, interquartile range (IQR=Q3-Q1) with 95% CI for median.

2.4.2 Hypotheses testing

The comparisons of data for five variables between the three study groups was done by the Kruskal-Wallis test because all data was skewed (not normally distributed). Sample sizes, medians, mean ranks, Kruskal-Wallis H, degree of freedom, and significance (p-value) are given for each test. In next step, pairwise comparisons are done giving us the significance of difference between the pairs; healthy controls and SH and healthy controls and OH, showing mean ranks, test statistic, standard error (SE) and p-value for each test at alpha 0.05. The data were analyzed by IBM SPSS for Windows, version 22.0 (IBM Corp., Armonk, New York).

3. RESULTS

3.1 Tests of Normality: Table 3.1 displays the results, along with an interpretation based on the five tests. All variables are interpreted as skewed.

3.2 Descriptive statistics & estimation of parameters: The mean ± SD age was 45.7±8.2 years for group 1 (control group), 47.1±6.6 years for group 2 subclinical hypothyroidism (SH), and 46.4±7.9 years for group 3 overt hypothyroidism (OH).

Table 3.1: Normality tests for serum levels of oxidative stress markers in individuals with no hypothyroidism (n1=45), subclinical hypothyroidism (n2=45), and overt hypothyroidism (n3=45) in Kutahya city, Turkey.

Variables	Groups	Skewness	Kurtosis	CV %	W	p-value	Data distribution
TOS (μmol H2O2 Eq./L)	Control	0.606	-0.181	28.78	0.961	.127	Normal
	SH	2.134	6.211	64.41	0.789	<.0001	Skewed
	OH	2.136	5.220	59.66	0.7807	<.0001	Skewed
TAS (mmol Trolox Eq./L)	Control	1.722	2.900	25.66	0.781	<.0001	Skewed
	SH	0.768	0.083	10.54	0.945	.032	Skewed
	OH	-0.425	-0.993	27.33	0.9335	.012	Skewed
OSI (Arbitrary Unit)	Control	1.108	1.722	37.36	0.915	.003	Skewed
	SH	0.834	0.853	35.23	0.935	.014	Skewed
	OH	1.314	1.803	42.64	0.888	.0004	Skewed
PON-1 (U/L)	Control	-0.269	-0.498	34.90	0.961	.129	Normal
	SH	0.558	-0.505	43.33	0.948	.042	Skewed
	OH	2.007	3.946	47.15	0.769	<.0001	Skewed
ARYL (U/L)	Control	0.154	-0.773	18.42	0.973	.358	Normal
	SH	-0.809	1.328	23.58	0.956	.087	Normal
	OH	-0.086	-1.414	29.14	0.922	.005	Skewed

CV%=Coefficient of variation %, W=Shapiro-Wilk statistics, SH=Subclinical hypothyroidism group, OH=Overt hypothyroidism group

Table 3.2 shows serum levels of oxidative stress markers in control, subclinical and overt hypothyroidism. The median serum levels of TOS and OSI are higher in SH and OH groups than control group, while those of TAS, PON-1 and ARYL are lower in SH and OH groups than control group.

3.3 Hypotheses Testing

3.3.1 TOS levels in control, SH & OH (H_{01} & H_{02}): The difference of median level of TOS between the control, SH and OH groups was testified by Kruskal-Wallis test. The difference was statistically significant with a p-value of <.0001. (Table 3.3.1.1)

Table 3.2: Serum levels of oxidative stress markers in individuals with no hypothyroidism (n1=45), subclinical hypothyroidism (n2=45), and overt hypothyroidism (n3=45) in Kutahya city, Turkey.

Variables	Groups	Sample statistics				95% CI for median	
		Quartile 1 (Q1)	Median (Q2)	Quartile 3 (Q3)	IQR	Lower	Upper
TOS (μmol H2O2 Eq./L)	Control	3.645	4.410	5.700	2.055	4.060	5.130
	SH	3.755	5.260	8.385	4.630	4.170	6.880
	OH	5.325	7.040	10.23	4.905	6.080	8.320
TAS (mmol Trolox Eq./L)	Control	1.670	1.800	2.425	0.755	1.700	1.870
	SH	1.455	1.540	1.685	0.230	1.490	1.620
	OH	0.890	1.250	1.385	0.495	1.010	1.340
OSI (Arbitrary Unit)	Control	0.220	0.300	0.390	0.170	0.250	0.360
	SH	0.390	0.520	0.693	0.303	0.416	0.636
	OH	0.475	0.640	0.890	0.415	0.540	0.740
PON-1 (U/L)	Control	253.5	325.0	372.5	119.0	282.0	349.0
	SH	144.0	172.0	263.5	119.5	154.0	237.0
	OH	100.5	116.0	143.5	43.0	103.0	131.0
ARYL (U/L)	Control	537.0	613.0	710.5	173.5	558.0	668.0
	SH	382.5	454.0	539.0	156.5	430.0	509.0
	OH	274.0	374.0	504.5	230.5	325.0	471.0

Q=Quartile, IQR=Inter quartile range (Q3-Q1), CI=Confidence Interval, SH= Subclinical hypothyroidism group, OH= Overt hypothyroidism group

Table 3.3.1.1: Serum levels of TOS in control, subclinical (SH) and overt hypothyroidism (OH) in population of Kutahya city, Turkey

Variable	Groups	N	Median	Mean Rank	Kruskal-Wallis H	d.f.	p-value (2-tailed)
TOS	Control	45	4.410	48.57	24.433	2	<.0001
(μmol H2O2 Eq./L)	SH	45	5.260	66.22			
	OH	45	7.040	89.21			

Q= Quartile, IQR = Interquartile range, d.f. = Degree of freedom

Multiple comparison tests revealed a statistically significant difference between the control and SH groups, as well as the control and OH groups, indicating that the median levels of TOS in SH and OH are much greater than the control group. (Table 3.3.1.2)

3.3.2 TAS levels in control, SH & OH (H_{03} & H_{04}): The difference of median level of TAS between the control, SH and OH groups was testified by Kruskal-Wallis test. The difference was statistically significant with a p-value of <.0001. (Table 3.3.2.1)

Multiple comparison tests revealed a statistically significant difference between the control and SH groups, as well as the control and OH groups, indicating that the median TAS levels in SH and OH are significantly lower than the control group. (Table 3.3.2.2)

3.3.3 OSI level in control, SH & OH (H_{05} & H_{06}): The difference of median level of OSI between the control, SH and OH groups were testified by Kruskal-Wallis test. The difference was statistically significant with a p-value of <.0001. (Table 3.3.3.1)

Table 3.3.1.2: Serum levels of TOS in subclinical (SH) vs. control and in overt hypothyroidism (OH) vs. control in population of Kutahya city, Turkey

Groups	N	Mean Rank	Test statistic	Stand. Error	p-value	Decision
Control group	45	48.57	-17.65	8.246	.032	H_{01} rejected
SH group	45	66.22				
Control group	45	48.57	-40.64	8.246	<.0001	H_{02} rejected
OH group	45	89.21				

Table 3.3.2.1: Serum levels of TAS in control, subclinical (SH) and overt hypothyroidism (OH) in population of Kutahya city, Turkey

Variable	Groups	N	Median	Mean Rank	Kruskal-Wallis H	d.f.	p-value (2-tailed)
TAS (mmol Trolox Eq./L)	Control	45	1.800	103.90	83.083	2	<.0001
	SH	45	1.540	71.16			
	OH	45	1.250	28.94			

Q= Quartile, IQR = Interquartile range, d.f. = Degree of freedom

Table 3.3.2.2: Serum levels of TAS in subclinical (SH) vs. control and in overt hypothyroidism (OH) vs. control in population of Kutahya city, Turkey

Groups	N	Mean Rank	Test statistic	Stand. Error	p-value	Decision
Control group	45	103.90	32.74	8.245	<.0001	H_{03} rejected
SH group	45	71.16				
Control group	45	103.90	74.96	8.245	<.0001	H_{04} rejected
OH group	45	28.94				

Table 3.3.3.1: Serum levels of OSI in control, subclinical (SH) and overt hypothyroidism (OH) in population of Kutahya city, Turkey

Variable	Groups	N	Median	Mean Rank	Kruskal-Wallis H	d.f.	p-value (2-tailed)
OSI (Arbitrary Unit)	Control	45	0.300	32.54	60.437	2	<.0001
	SH	45	0.520	76.54			
	OH	45	0.640	94.91			

Q= Quartile, IQR= Interquartile range, d.f = Degree of freedom

Multiple comparison tests revealed a statistically significant difference between the control and SH groups, as well as the control and OH groups, indicating that the median OSI levels in SH and OH are significantly higher than the control group. (Table 3.3.3.2)

3.3.4 PON-1 level in control, SH & OH (H_{07} & H_{08}): The difference of median level of PON-1 between the control, SH and OH groups was testified by Kruskal-Wallis test. The difference was statistically significant with a p-value of <.0001. (Table 3.3.4.1)

Multiple comparison tests revealed a statistically significant difference between the control and SH groups, as well as the control and OH groups, indicating that the median levels of PON-1 in SH and OH are significantly lower than the control group. (Table 3.3.4.2)

3.3.5 ARYL level in control, SH & OH (H_{09} & H_{010}): The difference of median level of ARYL between the control, SH and OH groups was testified by Kruskal-Wallis test. The difference was statistically significant with a p-value of <.0001. (Table 3.3.5.1)

Table 3.3.3.2: Serum levels of OSI in subclinical (SH) vs. control and in overt hypothyroidism (OH) vs. control in population of Kutahya city, Turkey

Groups	N	Mean Rank	Test statistic	Stand. Error	p-value	Decision
Control group	45	32.54	-44.00	8.245	<.0001	H_{05} rejected
SH group	45	76.54				
Control group	45	32.54	-62.37	8.245	<.0001	H_{06} rejected
OH group	45	94.91				

Table 3.3.4.1: Serum levels of PON-1 in control, subclinical (SH) and overt hypothyroidism (OH) in population of Kutahya city, Turkey

Variable	Groups	N	Median	Mean Rank	Kruskal- Wallis H	d.f.	p-value (2- tailed)
PON-1 (U/L)	Control	45	325.0	99.11	53.872	2	<.0001
	SH	45	172.0	66.22			
	OH	45	116.0	38.67			

Q= Quartile, IQR= Interquartile range, d.f. = Degree of freedom

Table 3.3.4.2: Serum levels of PON-1 in subclinical (SH) vs. control and in overt hypothyroidism (OH) vs. control in population of Kutahya city, Turkey

Groups	N	Mean Rank	Test statistic	Stand. Error	p-value	Decision
Control group	45	99.11	32.89	8.246	<.0001	H_{07} rejected
SH group	45	66.22				
Control group	45	99.11	60.44	8.246	<.0001	H_{08} rejected
OH group	45	38.67				

Table 3.3.5.1: Serum levels of ARYL in control, subclinical (SH) and overt hypothyroidism (OH) in population of Kutahya city, Turkey

Variable	Groups	N	Median	Mean Rank	Kruskal- Wallis H	d.f.	p-value (2- tailed)
ARYL (U/L)	Control	45	613.0	102.33	57.391	2	<.0001
	SH	45	454.0	60.40			
	OH	45	374.0	41.27			

Q= Quartile, IQR = Interquartile range, d.f. = Degree of freedom

Table 3.3.5.2: Serum levels of ARYL in subclinical (SH) vs. control and in overt hypothyroidism (OH) vs. control in population of Kutahya city, Turkey

Groups	N	Mean Rank	Test statistic	Stand. Error	p-value	Decision
Control group	45	102.33	41.93	8.246	<.0001	H_{09} rejected
SH group	45	60.40				
Control group	45	102.33	61.06	8.246	<.0001	H_{010} rejected
OH group	45	41.27				

Multiple comparison tests revealed a statistically significant difference between the control and SH groups, as well as the control and OH groups, indicating that the median levels of ARYL in SH and OH are significantly lower than the control group. (Table 3.3.5.2)

4. DISCUSSION

4.1 TOS levels in control, SH & OH (H_{01} & H_{02}): Our study showed that median serum levels of TOS (μmol H2O2 Eq./L) are significantly higher in the SH (5.260, p=0.032) and OH (7.040, p=<.0001) groups as compared to healthy control (4.410). (Table 3.3.1.2)

Studies focused on Hashimoto's thyroiditis (a different thyroid disorder) reported that serum level of TOS was increased.[26,27] Aydogdu, et al.[28] found that serum level of TOS was significantly higher in SH than healthy controls.

4.2 TAS levels in control, SH & OH (H_{03} & H_{04}): Our study showed that median serum levels of TAS (mmol Trolox Eq./L) are significantly lower in the SH (1.540, p=<.0001) and OH (1.250, p=<.0001) groups as compared to healthy control (1.800). (Table 3.3.2.2)

Ates, et al. have reported in their different studies that Hashimoto's thyroiditis patients had decreased serum TAS levels.[26,27] Aydogdu, et al.[28] indicated that serum level of TAS was lower in SH patients as compared to healthy controls.

4.3 OSI levels in control, SH & OH (H_{05} & H_{06}): Our study showed that median serum levels of OSI (Arbitrary Unit) are significantly higher in the SH (0.520, p=<.0001) and OH (0.640, p=.0001) groups as compared to healthy controls (0.300). (Table 3.3.3.2)

Oxidative stress is measured by investigating numerous oxidative stress markers, including TOS and TAS. Many researchers have indicated in prior studies that OSI is increased in Hashimoto's thyroiditis[26,27] and SH patients[28] when compared to healthy controls.

4.4 PON-1 activities in control, SH & OH (H_{07} & H_{08}): Our study showed that median serum activities of PON-1 (U/L) are significantly lower in the SH (172.0, p=<.0001) and OH (116.0, p=<.0001) groups as compared to healthy controls (325.0). (Table 3.3.4.2)

PON-1, an antioxidant enzyme on HDL-cholesterol, is known to protect HDL-cholesterol and LDL-cholesterol against peroxidation caused by free radicals.[17] Serum PON-1 activities have previously been found to be lower in SH patients compared to healthy controls.[18,29,30]

The mechanism of lower PON-1 activity in hypothyroidism is still unclear; it is thought that it might be a result of increased oxidized lipid levels and ROS, which inhibit PON-1 activity. Furthermore, it has been reported that PON-1 activity is also reduced in a variety of metabolic disorders as a result of ROS pathogenesis associated with oxidative stress and inflammation.[18]

4.5 ARYL activities in control, SH & OH (H_{09} & H_{010}): Our study showed that median serum activities of ARYL (U/L) are significantly lower in the SH (454.0, p=<.0001) and OH (374.0, p=<.0001) groups as compared to healthy controls (613.0). (Table 3.3.5.2)

ARYL, a PON-1-related antioxidant enzyme and oxidative stress marker, was found significantly lower in SH patients than in healthy subjects.[29,30]

5. CONCLUSIONS

Our study showed that serum levels of TOS and OSI were higher in subclinical and overt hypothyroidism than in healthy controls; while serum levels of TAS, PON-1, and ARYL were lower in subclinical and overt hypothyroidism than in healthy controls.

The oxidative stress profile is increased and the antioxidant defense is weakened in patients with both subclinical and overt hypothyroidism. Reduced PON-1 and ARYL activities can lead to a lack of protection against lipid peroxidation and DNA oxidative damage, exposing patients at risk for future cardiovascular problems or cancer.

As things stand, we believe that oxidative stress and an unbalanced oxidant/ antioxidant defense are serious health issue in hypothyroidism, with the potential to induce a variety of illnesses, some of which are life-threatening. On the basis of these findings, we can conclude that OH and SH are linked

to an increase in oxidative stress. Overproduction of reactive oxygen species (ROS) and a lack of antioxidant defense contribute to oxidative stress, a critical stage in metabolic disorders that can lead to mortality. Further studies in larger populations are needed to better understand the prognosis and risk factors of hypothyroidism, including age, sex, ethnicity, regional variations, nutritional habits, and so on.

Acknowledgement: We highly acknowledge the grant of permission by Dr. Muhammad Marwat (marwatmuhammad@ gmail.com) from Gomal Medical College, D.I.Khan, Pakistan to use his innovated "Marwat's Logical Trajectory of Research Process" in our project and further his help in data analysis and report writing."

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

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Acquisition, Analysis or Interpretation of Data:	MY, SA, BSE, FEK
Manuscript Writing & Approval:	MY, SA, BSE, FEK

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.