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# Status of Serum Levels of Oxidative Stress **Biochemical Markers and Total Antioxidant** Capacity in Primary Hypothyroidism

Abbas Sabbar Jebur\*<sup>1</sup>, Basil Oied Mohammed Saleh<sup>2</sup>, Omar Farooq Nafea Al-Azzawi<sup>3</sup>

#### **Abstract**

**Background:** Primary hypothyroidism (HT) has been demonstrated to be associated with oxidative stress. This study was designed to assess the role of oxidative stress in the pathogenesis of primary hypothyroidism.

Methods: The study included 97 subjects, age range (of 29-62 years); 57 of them had been diagnosed with primary hypothyroidism, and 40 healthy subjects as controls in Baghdad, during Oct 2023 to 2024. The primary HT subjects were sub-classified into the newly diagnosed primary HT group (n=24) and the established primary HT (n=33) group. Investigations encompassed serum evaluation of total antioxidant capacity (TAC), total oxidant status (TOS), 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), NADPH oxidase-4 (NOX4), and Anti-TPO utilizing enzymatic colorimetric methods and enzyme-linked immunosorbent assay (ELISA).

**Results:** The median and 1<sup>st</sup> -3<sup>rd</sup> quartile range values of serum 8-oxo-7,8-dihydro-2'-deoxyguanosine, NADPH oxidase-4, and total antioxidant capacity levels of newly diagnosed and established primary HT were significantly elevated when correlated with those of controls (for all, p < 0.0001), with nonsignificant differences between both groups of primary HT. The reservoir operating characteristic (ROC) and area under curve revealed that both total oxidant status and DNA damage 8-oxo-dG had high sensitivity and specificity in differentiation between hypothyroidism patients and controls at defined

Conclusion: Elevated levels of serum 8-oxodG, NOX4, and TOS reflect the underlying oxidative damage associated with reduced thyroid function and may participate to the pathogenesis of primary hypothyroidism.

**Keywords:** Antioxidant status, Oxidative Stress, Primary hypothyroidism.

### Introduction

An essential function of thyroid hormones triiodothyronine (T3) and thyroxine (T4) is to modify the metabolic rate of specific tissues, including the kidneys, heart, brain, and liver (1,2). There is a high prevalence of thyroid function abnormalities among the overall population. (3,4). Primary hypothyroidism is usually caused by autoimmune thyroiditis and goiter. A positive thyroid autoantibody antithyroid peroxidase test confirms the diagnosis

Hypothyroidism (HT) has been demonstrated to have a higher level of oxidative stress (OS), an imbalance in the the equilibrium that favors increased production of free radicals and a reduction in antioxidants (6,7). However, the results are still scarce and somewhat controversial (8). It is commonly acknowledged that inflammation has connections to OS (9).

According to earlier research, guanine is the

<sup>1,2:</sup> Department of Clinical Biochemistry, College of Medicine, University of Baghdad, Baghdad, Iraq.

<sup>3:</sup> Department of Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq.

most oxidized of the four bases that form the DNA structure (10). The oxidized form of 8hydroxy-2'-deoxyguanosine (8-OHdG) or 8oxo-7. 8-dihydro-2'-deoxyguanosine oxodG) acts as one of the most significant biological oxidative stress (OS) markers. It is the most sensitive and relevant marker of oxidative DNA damage (11,12), which is prevalent in various tissues and fluids due to its ability to permeate from cells into the bloodstream readily (12). Nonetheless, the biological function of 8-OHdG remains inadequately comprehended (13,14). NADPH oxidases (NOXs) are a component of the NOX family of enzymes, serving as the catalytic subunit within the NADPH oxidase complex. This family serves as proficient producers of reactive oxygen species (ROS), with their principal role being the generation of these molecules (15). In contrast to dual oxidases (DUOXs), NOX4 is one of the members of the NOX family that is highly expressed in the thyroid and regulated transcriptional level by thyroid-stimulating hormone (16,17).(TSH) heterodimerization of NOX4 with p22phox enhances the production of reactive oxygen species (ROS) (18). Antioxidants are small molecular weight substances found in plasma, including vitamin C, vitamin E, bilirubin, and uric acid (19). Total antioxidant capacity serves as the principal metric for assessing oxidative stress levels and potential in aging and related diseases (20–22). This study aimed to investigate the oxidative stress in the pathogenesis of primary hypothyroidism, propose a new diagnostic approach, and compare it with healthy subjects.

#### Subjects and methods

This case-control study was performed at the Specialized Centre for Endocrinology and Diabetes, Medical City, Baghdad, Iraq, by the Department of Biochemistry, College of Medicine, University of Baghdad, from October 2023 to May 2024. The study involved 97 subjects; 57 were diagnosed with primary hypothyroidism (HT) by a specialist endocrinologist, and 40 healthy subjects

served as controls, free from any acute or chronic illnesses. The healthy controls were selected from colleagues and relatives from Baghdad and other governments after detailed inquiries about their medical history. Inclusion criteria included those patient who have had primary HT and were sub-classified according to the time of disease diagnosis into the 'newly diagnosed primary HT group' (duration of disease within a week of the study inclusion and before starting thyroxine treatment), which included 24 patients, and the 'established primary HT group' (those patients who were already on thyroxine treatment) included 33 patients. The diagnosis of primary hypothyroidism was established using clinical examination, radiographic assessments, and thyroid function testing, including serum TSH, tetraiodothyronine free (fT4). free triiodothyronine (fT3), and anti-thyroid peroxidase (anti-TPO) to confirm Hashimoto's thyroiditis.

The cases and controls were selected at the age range of 29 to 62 years. The ethical and scientific review boards of the University of Baghdad's College of Medicine and Department of Biochemistry gave their stamp of approval to this study. Additionally, the Ministry of Health of Iraq and the Specialized Center for Endocrinology and Diabetes at Medical City in Baghdad provided their ethical clearance. Participants gave their verbal consent before participating in this study. The control group consisted of 40 healthy subjects selected from colleagues and relatives who were healthy and not suffering from thyroid disorders or any acute or chronic conditions.

exclusion criteria encompassed patients with diabetes mellitus, alcohol abuse, smoking, pregnancy, cardiovascular diseases, renal disease, secondary hypothyroidism, tumors, liver diseases, and any other acute or chronic illnesses identified through medical history, physical examination, and laboratory results.

Serum markers analysis

Five milliliters (ml) of blood were drawn from the peripheral veins of each patient and control group, allowed to clot for 15 minutes,

and subsequently centrifuged for 10 minutes at 2500 rpm. TSH, fT4, fT3, Anti-TPO, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), NADPH oxidase-4 (NOX4), total antioxidant capacity (TAC), and total oxidant status (TOS) was measured in the separated serum, which was kept at -45 °C until the day of laboratory testing. The thyroid function tests (TSH, fT4, fT3) were assayed enzymatically on a fully automated **MAGLUMI®** Chemiluminescence Immunoassay (CLIA) System (United Kingdom). The principle of CLIA, specifically utilizing an acridinium aster-based immunoassay (ABEI) technology. This technique is characterized by its high specificity, sensitivity and making appropriate for a wide range of diagnostic tests. Measurements of TAC and TOS were performed using enzymatic colorimetric methods (Elabscience, USA). While measurements of (8-oxodG), NOX4, and Anti-TPO were achieved by using enzyme-linked immunosorbent assay (ELISA) sandwich methods (Elabscience, USA). The principle of (Enzyme-Linked sandwich **ELISA** Immunosorbent Assay) involves the use of two different antibodies that bind to distinct epitopes on the same antigen.

# Statistical analysis

Data analysis was performed using SPSS version 26 software to calculate the mean, standard deviation, median, and the interquartile range (Q1-Q3) for the data

analysed in this study. ANOVA was used to assess the variations between the means of numeric data. Considering the data that did not meet the assumptions of normality required for parametric tests, to compare the medians of three groups, the Kruskal-Wallis test, a nonparametric alternative to one-way ANOVA, was used to compare the medians of three groups. When significant differences were found, a Bonferroni-adjusted Mann-Whitney post hoc test was used to determine which groups differ. The cutoff value, specificity, and sensitivity of the parameters have been determined for differentiation among the three hypothyroidism groups and the control group. Additionally, receiver operating the characteristic (ROC) curve and area under the curve (AUC) were analyzed. Pearson correlation coefficient (r) was used to assess the correlation between numeric variables. A p-value less than 0.05 was considered statistically significant.

#### Results

The mean and standard deviation values of age and body mass index (BMI) for the three groups under investigation, newly diagnosed primary HT, established primary HT, and controls, are presented in Table 1. The mean age values did not differ significantly among the groups. However, the newly diagnosed HT group had a significantly higher BMI than controls.

**Table 1.** Mean (±SD) values of age and body mass index in newly diagnosed primary hypothyroidism, established primary hypothyroidism and control groups.

Parameter	Control (n=40)	Newly diagnosed primary HT (n=24)	Established primary HT (n=33)
Age (years) <sup>NS</sup>	41.93±8.39	42.46±11.03	45.79±14.26
Body mass index (Kg/m <sup>2</sup> )	27.61±2.95	30.6±4.29 <b>•</b>	30.23±5.48 <sup>NS</sup>

ANOVA and t-test indicated: NS: no significant variance within or between groups.

• Significant rise in BMI in newly diagnosed hypertension compared to controls.

The median values of serum TSH and were significantly increased in both the newly diagnosed and established primary HT groups compared to controls (p< 0.001) (Table 2). Likewise, the median serum TSH level in

the newly diagnosed primary HT group was significantly higher than that in the established primary HT group (p< 0.0001). However, there was no statistically significant difference in the median fT3 levels among the three

groups. On the other hand, the median values of fT4 were significantly decreased in the newly diagnosed primary HT group compared to both controls and the established primary HT (p< 0.001 for both), with a non-significant difference between the control and established primary HT. The median value of anti-TPO

levels was significantly higher in the established primary HT than in controls (p< 0.019), but there were no significant differences between the newly diagnosed primary HT and control groups, nor between the newly diagnosed and established primary HT groups.

**Table 2.** Median (1st -3rd quartile range) values of TSH, fT3, fT4, and anti-TPO levels in newly diagnosed primary

hypothyroidism, established primary hypothyroidism and control groups.

Parameter	Control average and range (n=40)	Newly diagnosed primary HT range (n=24)	Established primary HT range (n=33)
TSH (uIU/ml)	2.21	22.07•,••	6.00 <b>°</b>
	(1.41-2.67)	(18.4-63.01)	(3.18-14.6)
fT3 (pg/ml)	2.79	2.66	2.85
	(2.4-3)	(2.38-2.82)	(2.67-3.08)
fT4 (ng/dl)	1.08	0.6*	$1.07^{ m NS}$
	(1.05-1.15)	(0.54-0.64)	(1.05-1.13)
Anti- thyroid			
peroxidase	17.33	$18.75^{NS}$	19.03 <b>*</b>
antibodies	(15.73-18.02)	(17.15-23.71)	(16.01-21.21)
(ng/ml)			

Kruskal-Wallis and Bonferroni-adjusted Mann-Whitney post-hoc analysis test revealed;  $\bullet$  significant increase in TSH in newly diagnosed and established HT than in controls (for both, p=0.001),  $\bullet$  significant increase in TSH in newly diagnosed than in established HT (p=0.001),  $\bullet$  significant decrease in fT4 in newly diagnosed than in controls and established HT (p=0.001),  $\bullet$  significant increase in anti-TPO in established HT than in controls (p=0.019), NS: non-significant differences among and between groups in fT3, between established HT and controls in fT4, between newly diagnosed HT and each of controls and established HT in anti-TPO.

The median values of 8-oxodG, NOX4, and TOS levels of newly diagnosed and established primary HT were significantly higher compared to those of the control group (p < 0.0001 for all), with no significant differences between the two primary HT

groups (Table 3). However, the median values of TAC among the three studied groups, newly diagnosed HT, established HT, and controls, did not differ significantly among and between them.

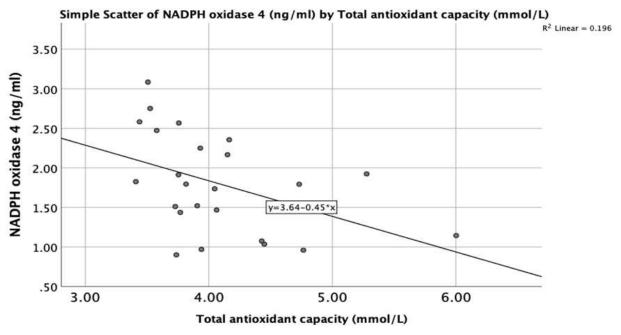
**Table 3.** Median (1<sup>st</sup> -3<sup>rd</sup> quartile range) values of 8-oxo-7,8-dihydro-2'- deoxyguanosine, NADPH oxidase-4, total antioxidant capacity and Total oxidant status levels in newly diagnosed primary hypothyroidism, established primary hypothyroidism and controls groups.

Parameter	Control average and range (n=40)	Newly diagnosed primary HT(n=24)	Established primary HT (n=33)
8-oxo-7, 8-dihydro- 2'-	38.29	60.72 <sup>♦, NS</sup>	62.85*
deoxyguanosine (ng/mL)	(26.79-44.76)	(59.28-63.5)	(61.97-64.32)
NADPH oxidase 4	1.08	1.79 <sup>♦, NS</sup>	1.8*
(ng/mL)	(1-1.15)	(1.47-2.17)	(1.43-2.26)
Total oxidant status	38.21	85.37 <sup>♦, NS</sup>	90.71♦
(mmol/L)	(37.43-39.78)	(78.49-96.1)	(87.13-100.72)
Total antioxidant capacity	4.02	3.92	4.02
(mmol/L) <sup>NS</sup>	(3.87-4.12)	(3.75-4.15)	(3.8-4.22)

Kruskal-Wallis and Bonferroni-adjusted Mann-Whitney post-hoc analysis test revealed; ◆significant increase in 8-oxo-7, 8-dihydro- 2'-deoxyguanosine, NADPH oxidase4, total oxidant status in newly diagnosed and established HT than in controls (for all, *p*=0.001), NS: non-significant differences among and between groups in total antioxidant capacity, between newly diagnosed and established HT in 8-oxo-7, 8-dihydro- 2'-deoxyguanosine, NADPH oxidase4, total oxidant status.

In newly diagnosed primary HT, significant negative correlations were observed between TAC and NADPH oxidase (r=-0.471, p=0.02,

Fig. 1) as well as between 8-oxodG and fT3 (r= -0.468, p=0.021, Fig. 2).



**Fig. 1.** Scatter blot showing correlation between NADPH oxidase 4 and total anti-oxidant capacity in newly diagnosed primary hypothyroidism.

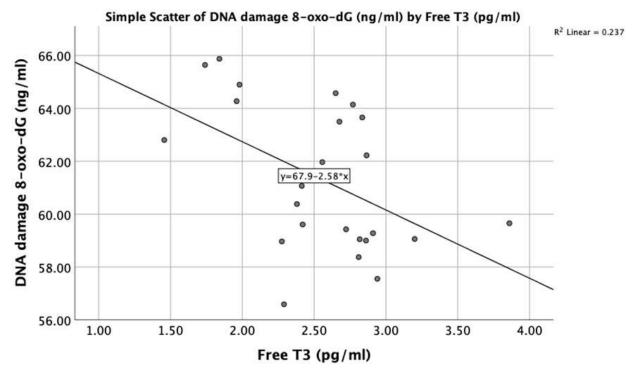


Fig. 2. Scatter blot showing the correlation between DNA damage 8-oxo-dG and free T3 in newly diagnosed primary hypothyroidism.

The receiver operating characteristic (ROC) and area under the curve (AUC) analysis revealed that both total oxidant status (TOS) at a cutoff of 50 mmol/l and DNA damage marker 8-oxo-dG at cutoff of 53 ng/ml showed full area under the curve (AUC=1.0) with 100% sensitivity and 100% specificity in differentiating between newly diagnosed primary HT and controls. Similarly, TOS at 50 mmol/L and 8-oxo-dG at 53 ng/mL also demonstrated AUC= 1.0. with 100% sensitivity and 100% specificity in distinguishing established primary HT from controls. However, the thyroidstimulating hormone (TSH) at cutoff of 3.5 uIU/ml showed an area under the curve (AUC) of 0.82 with 36.4% sensitivity and specificities differentiating between newly diagnosed and established primary HT.

#### Discussion

The established primary HT and control groups did not significantly differ in age or BMI; however, the newly diagnosed HT group had a significantly higher than the controls. In addition to fat accumulation, a deficiency in thyroid hormones leads to a decreased metabolic rate, resulting in reduced energy expenditure even at rest. Newly diagnosed hypothyroid patients often experience fluid retention due to altered kidney function and sodium retention, which is influenced by hormonal changes. This can contribute significantly to an increase in BMI, which is comparable with previous studies (23,24). Our study showed that thyroid function parameters, TSH and Free T4, increased dramatically in newly diagnosed primary hypothyroidism patients compared with the healthy control. The elevated TSH levels in patients with newly diagnosed hypothyroidism are a physiological response to low circulating levels of thyroid hormones. The pituitary gland senses the deficiency and compensates by secreting more TSH, which is indicative of the body's effort to restore homeostasis (25).The demonstrated that the concentration of 8-oxo-7.

8-dihydro-2'-deoxyguanosine was markedly elevated in both newly diagnosed and established primary hypothyroidism compared to healthy individuals. This finding aligns\*\* with previous studies conducted by Riis et al. (26) and Halczuk et al. (27). DNA damage (8oxo-dG) is a biomarker for oxidative stress, representing DNA damage caused by reactive oxygen species (ROS). Elevated levels of this compound indicate increased oxidative DNA damage, which can be a contributing factor in various diseases, including thyroid disorders. The thyroid gland is particularly susceptible to oxidative stress due to its high levels of hydrogen peroxide production during hormone synthesis, which can lead to the formation of 8oxodG when ROS levels are elevated. Previous research on Danish women with newly hypothyroidism diagnosed indicated urinary excretion of 8-oxodG was significantly elevated in comparison to healthy controls. This indicates an association between diminished thyroid hormone levels and elevated oxidative damage. (26). Our study demonstrated a significantly higher NADPH oxidase-4 in hypothyroid patients (both newly diagnosed and established) compared to the control group. NADPH oxidase-4 is a member of the NADPH oxidase family; NOX4 is responsible for producing superoxide and hydrogen peroxide, which are key reactive oxygen species (ROS) involved in cellular signaling and homeostasis. The excessive generation of reactive oxygen species (ROS) by NOX4 can disrupt normal thyroid function, influencing the production of thyroxine (T4) and triiodothyronine (T3). This disruption may contribute to the development of hypothyroidism by impairing the function of thyrocytes and leading to cellular apoptosis or dysfunction (28, 29). The expression of genes unique to the thyroid, such as thyroperoxidase (TPO) and the sodium/iodide symporter (NIS), is negatively correlated with NOX4 expression. Increased NOX4 activity can lead to reduced expression of these genes, which are necessary for the absorption of iodide and the production of hormones (30–32).

This study demonstrates that patients with hypothyroidism have significantly elevated

levels of total oxidant status (TOS) compared to healthy individuals. **Hypothyroidism** with is often associated chronic inflammation. contribute which may to increased production of reactive oxygen (ROS) by immune species cells. This heightened oxidative activity could explain the observed rise in TOS levels (33). In addition, this research indicates that the concentration of total antioxidant capacity (TAC) is not statistically significant among The three groups. current disagrees with previous research, which showed that TAC levels were significantly lower in individuals with hypothyroidism compared to healthy controls (36). This may be due to lifestyle differences in the population, treatment management, and sample size.

The elevated levels of serum 8-oxo-7,8-dihydro-2'- deoxyguanosine, NADPH oxidase 4 and total oxidant status (TOS) reflect the underlying oxidative damage associated with reduced thyroid function and may contribute to the pathogenesis of primary hypothyroidism.

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# **Conflict of interest**

The entire work had permitted by ethical committees of local authorities (429). All participants provided an inscribed informed consent, and the research was conducted in line with the ethical morals identified in the 1975 Treaty of Helsinki. The authors declare no potential conflicts of interest related to the present research.

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