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Evaluation of Cyclin-Dependent Kinase 4 (CDK4) in the Serum of Thyroid Cancer Patients and Its Association with Oxidative and Antioxidative Factors

*[1]Abdulazeez Ghalib Jumaa, [1]Eman A. Hadi

About Article

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About Author

[1] Department of Chemistry, College of Science, University of Mosul, Mosul, Iraq

Contact @ Abdulazeez Ghalib Jumaa
abdalazez.24scp66@student.uomosul.edu.iq

ABSTRACT

The study addressed key medical aspects, particularly the biochemical variables in patients with early-stage (Stage I and II) thyroid cancer, by analyzing blood serum samples. 130 samples were collected from patients in the range of 30 to 70 years. Among them, 60 patients were diagnosed as having papillary thyroid carcinoma by specialists and were undergoing treatment at the Oncology and Nuclear Medicine Hospital, while 70 were healthy individuals who served as controls. The subjects were divided into four groups: 30–39, 40–49, 50–59, and 60–70 years. They were also segregated into two groups based on the severity of the disease: Stage I and Stage II. Biochemical factors investigated included Cyclin-Dependent Kinase 4 (CDK4), uric acid, vitamin E, malondialdehyde (MDA), glutathione (GSH), and peroxynitrite ($ONOO^-$). Linear correlation test was employed to find out if there was a correlation between CDK4 and oxidative/antioxidative parameters in the patient and control groups. The results revealed greater concentrations of CDK4, MDA, and peroxynitrite in the patient group compared with the control group. Glutathione, vitamin E, and uric acid concentrations, on the other hand, were found to significantly reduce. In the control group, statistically significant relationships were established between CDK4 and all variables (uric acid, GSH, MDA, vitamin E, and $ONOO^-$), with positive relationships with antioxidants and negative relationships with oxidative stress markers. Among patients following thyroidectomy, significant negative correlations were found between CDK4 and both uric acid and MDA, while a positive correlation was observed between CDK4 and vitamin E. No significant correlations were found between CDK4 and either glutathione or peroxynitrite, which may indicate a disruption in oxidative balance post-surgery.

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1. INTRODUCTION

Thyroid cancer refers to the abnormal and uncontrolled thyroid cell growth in the thyroid gland that results in the formation of tumors, which could be benign or malignant. Unlike benign tumors, which tend to be localized and can be treated, malignant tumors can invade surrounding tissues and travel to distant organs via the lymphatic system or bloodstream. This cancer most frequently arises from follicular cells that produce thyroid hormones or parafollicular "C cells" that produce calcitonin, as seen in medullary thyroid carcinoma (Lloyd *et al.*, 2017; WHO, 2022; Ahmed *et al.*, 2022).

Cyclin-dependent kinase 4 (CDK4) is an important cell cycle regulating kinase. It partners with cyclin D to form the active CDK4/cyclin D complex, which phosphorylates the retinoblastoma protein (Rb), thereby enabling the cell to advance from the G1 to the S phase, where DNA replication occurs. The CDK4/6–Cyclin D–Rb pathway is at the core of the control of cell division and is tightly controlled in normal tissues. However, its dysregulation has been involved in tumorigenic alterations in a wide range of cancers, including thyroid cancer (Reddy *et al.*, 2005).

Antioxidants are vital biological molecules that protect the cells of the body from oxidative stress-induced injury—a process that occurs due to the accumulation of free radicals. Free radicals are reactive molecules that are created by normal metabolic processes or obtained from external sources such as ultraviolet radiation, environmental pollution, and an unhealthy diet. Free radicals have the potential to damage cellular DNA, proteins, and lipids (Al-Helaly *et al.*, 2012).

Scientific studies indicate that oxidative stress plays a critical role in the development of most diseases such as cancer, diabetes, cardiovascular disease, and neurodegenerative diseases (Halliwell & Gutteridge, 2015; Fathi *et al.*, 2023). In thyroid cancer, oxidative stress enhances the development of the disease by inducing genetic mutations, genomic instability, and facilitating the growth of malignant cells. Antioxidants, such as glutathione, superoxide dismutase, catalase, and vitamins C and E, exert protective roles by decreasing oxidative damage and reducing its harmful effects. It has been shown that defective antioxidant defense mechanisms or low levels of antioxidants may be linked to increased risk of thyroid cancer, especially differentiated types such as papillary and follicular carcinoma (Sies, 2020).

On the other hand, oxidants are unstable molecules characterized by unpaired electrons (free radicals), which drive them to react with cellular components to achieve stability. When not adequately regulated, these reactive species trigger oxidative stress, a central mechanism underlying genetic mutations and cellular alterations associated with tumor development (Valko *et al.*, 2007). Prominent oxidative agents include peroxynitrite ($ONOO^-$) and malondialdehyde (MDA).

Thus, understanding the dynamic between oxidative and antioxidative factors not only clarifies the mechanisms underlying disease progression but also opens pathways for utilizing biomarkers such as MDA and superoxide dismutase for early diagnosis, monitoring disease progression, and evaluating therapeutic efficacy.

2. LITERATURE REVIEW

Cyclin-Dependent Kinase 4 (CDK4) is a key serine/threonine kinase that regulates the cell cycle, particularly the transition from the G1 to the S phase through phosphorylation of the retinoblastoma (Rb) protein. Dysregulation and overexpression of CDK4 have been implicated in various cancers, including breast, lung, and thyroid malignancies, where it contributes to uncontrolled cellular proliferation and tumor progression (Zhang *et al.*, 2021; Liu *et al.*, 2023). In thyroid cancer, particularly papillary thyroid carcinoma, increased CDK4 expression has been correlated with more aggressive tumor phenotypes and poor clinical outcomes. This makes CDK4 not only a potential biomarker of prognosis but also a possible target for therapeutic intervention.

In parallel, oxidative stress has been established as a significant contributing factor in the initiation and progression of cancer. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological antioxidant systems to neutralize these reactive intermediates. One of the primary biomarkers used to assess lipid peroxidation caused by oxidative stress is malondialdehyde (MDA). Elevated MDA levels have been reported in various malignancies, including thyroid cancer, indicating an increased oxidative burden in these patients (Akinci *et al.*, 2008; Kościuszko *et al.*, 2023). These elevated levels are often linked to cellular membrane damage and the promotion of oncogenic signaling pathways.

Superoxide Dismutase (SOD) is one of the key antioxidant enzymes responsible for the detoxification of superoxide radicals by converting them into molecular oxygen and hydrogen peroxide. Alterations in SOD activity have been associated with numerous pathological states, including cancer. In thyroid cancer, studies have shown variable activity of SOD, with some reporting reduced enzymatic activity, which reflects compromised antioxidant defense and contributes further to tumor progression (Kumar & Gupta, 2021). This decline in antioxidant defense mechanisms enhances cellular susceptibility to oxidative DNA damage, mutations, and further malignant transformation.

Despite the extensive literature describing the roles of CDK4, MDA, and SOD independently, few studies have integrated these markers within the same investigative framework, especially in the context of thyroid cancer. Most research has tended to focus on these biomarkers in isolation, failing to explore potential interconnections between cell cycle regulation and oxidative stress pathways. Understanding how CDK4 expression correlates with oxidative (MDA) and antioxidative (SOD) biomarkers could provide valuable insights into the complex interplay between cell proliferation and redox homeostasis in thyroid cancer pathogenesis. Furthermore, such integrated evaluations may open new avenues for the development of combination biomarkers to improve diagnostic accuracy and patient management strategies in clinical oncology.

3. METHODOLOGY

This study involved a total of 130 blood samples collected from individuals diagnosed with thyroid cancer as well as from healthy controls, during the period from October 2024 to

March 2025, in collaboration with the Oncology and Nuclear Medicine Hospital in Mosul. Among these, 60 blood samples were obtained from patients diagnosed with papillary thyroid carcinoma, with ages ranging between 30 and 70 years. In addition, 70 samples were collected from apparently healthy individuals within the same age range (30–70 years).

The experimental work was conducted in the laboratories of the Department of Chemistry, College of Science, University of Mosul, as well as in private diagnostic laboratories. Blood samples were collected by venipuncture, drawing 5 mL of whole blood from each participant. The serum was separated and stored at −20 °C until biochemical analysis was performed (Burits *et al.*, 2015).

For the measurement of cyclin-dependent kinase 4 (CDK4) in serum, ready-to-use ELISA kits from BT Lab Bioassay Technology Laboratory (China) were employed. Uric acid levels were measured using commercial kits from Roche (Germany), specifically with the Elecsys and cobas e411 systems. Other biochemical variables were determined using conventional manual methods with reagents sourced from various companies, including Sigma (USA), Fluka (Sweden), and Hopkins & Williams (UK).

3.1. Statistical analysis

The data were statistically analyzed using SPSS version 26. One-way analysis of variance (ANOVA) was applied to determine the mean ± standard deviation (Mean ± SD) for the studied variables. Pearson's correlation coefficient (r) was used to assess linear relationships among variables. Comparisons between groups were conducted using Duncan's multiple range test. Statistical significance was considered at a p-value ≤ 0.05 (Salim & Adnan, 2017).

4. RESULTS AND DISCUSSION

4.1. Cyclin Dependent Kinase 4 (CDK4)

4.1.1. CDK4 Enzyme activity and its implications in thyroid cancer

The activity of cyclin-dependent kinase 4 (CDK4) in the healthy control group was 3.18 ng/ml, while a statistically significant increase in CDK4 activity was observed in thyroid cancer patients, reaching 4.72 ng/ml.

CDK4 was quantified in the patients as well as the controls, and the differences within the same age groups between the control group and the different stages of cancer were as follows:

- A significant increase in the activity of CDK4 ($p \leq 0.05$) was observed among the control and Stage I thyroid cancer patients.
- Additionally, a notable rise ($p \leq 0.05$) was observed in Stage I and Stage II patients across every age group.

The overexpression of CDK4 in patients with thyroid cancer is linked with dysregulation of the cell cycle. It is due to hyperactivation of the CDK4/Cyclin D pathway, which is usually brought about by overexpression of proliferation-inducing genes or loss of critical inhibitors of the cell cycle such as p16^INK4a, resulting in the uncontrolled proliferation of cancer cells (Malumbres & Barbacid, 2009). The cancer cells become increasingly aggressive and proliferative as the disease develops. Each of these steps during tumor development is accompanied by accumulating genetic alterations that inactivate normal brakes on cell proliferation. As a result, CDK4 levels rise further to allow rapid tumor expansion.

This finding is consistent with the results reported by (Pita *et al.* 2023), who demonstrated that activated CDK4 levels are significantly elevated in various types of thyroid carcinoma compared to normal thyroid tissues. Moreover, microscopic metastatic cells or active cancer stem cells may persist even after surgical thyroidectomy, particularly in cases where adjuvant therapies—such as radioactive iodine treatment—are not administered, which sustains the elevated expression of CDK4. Additionally, chronic inflammation induced by the tumor itself or as a result of surgical intervention may stimulate the tumor microenvironment and contribute to the upregulation of CDK4 (Hanahan & Weinberg, 2011).

Table 1. Different letters indicate a statistically significant difference at the $p \leq 0.05$ level, while identical letters suggest no significant difference.

Age groups (Year)	(CDK4 ng/ml) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	3.68±0.367 N=18 d	4.92±0.392 N=16 bc	5.46±0.338 N=4 a
Group 2 (40-49)	3.24±0.355 N=17 e	4.59±0.520 N=13 c	5.15±0.692 N= 7 ab
Group 3 (50-59)	2.97±0.319 N=24 ef	4.02±0.504 N=5 dc	4.86±0.214 N= 7 b
Group 4 (60-70)	2.72±0.270 N=11 f	3.70±0.311 N=4 d	4.52±0.374 N=4 bc

4.2. Differences in CDK4 Activity across Age Groups

There was a significant decline in CDK4 activity from age group one to two in the control group ($p \leq 0.05$), whereas that of second and third and third and fourth age groups were not significant. This is due to the fact that the first age group is the late phase of youth, where cell division and replenishment are at their peak, and the second age group is the start of middle age, where cellular turnover will begin to decline.

In patients in both Stage I and Stage II of thyroid carcinoma, variation of CDK4 activity across different age groups was not statistically significant. This means that cancer-induced increase of CDK4 activity would be capable of overcoming the age-related decrease.

At Stage II in particular, CDK4 activity did not vary statistically across all age groups.

In general, CDK4 activity reduces with increasing age as a result of reduced cellular proliferation and regeneration. This results from increased expression of natural inhibitors such as

p16^INK4a, which inhibit CDK4 activity. Aging cells are also less responsive to growth factor stimulation and can assume a state of cellular senescence, in which the cells do not divide but remain alive. These combined effects explain the reduced levels of CDK4 with advancing age (Krishnamurthy *et al.*, 2004).

4.3. Uric Acid

Age Group Variations in Uric Acid Concentration: A significant decrease in uric acid concentration ($p \leq 0.05$) was observed between the control group and Stage I thyroid cancer patients across all age groups.

A non-significant decrease was noted in uric acid levels between Stage I and Stage II patients in all age groups.

Several studies have shown that thyroidectomy leads to a reduction in overall metabolic activity, particularly in the early postoperative phase. This impacts the oxidative balance in the body. Uric acid, a potent antioxidant in plasma and the end product of purine metabolism, is believed to increase in cancer as a compensatory response to elevated oxidative stress. After tumor removal, oxidative stress decreases, reducing the body's need for endogenous antioxidants such as uric acid.

This observation aligns with findings by (Hu *et al.* 2021), who reported a decline in uric acid levels following thyroid tumor resection, suggesting that this reflects a reduction in oxidative stress after surgery. The absence of a clear difference between Stage I and Stage II further supports the notion that uric acid levels are more closely related to postoperative metabolic changes rather than tumor progression.

Across different age groups, a non-significant increase in uric acid levels was observed in the control group as well as in Stage I and II patients. These results indicate that age alone does not significantly influence uric acid concentrations, particularly in the absence of chronic conditions. This conclusion is supported by (Johnson *et al.* 2014,) who noted that while age may be weakly associated with uric acid levels in some cases, the effect is typically non-significant in healthy or medically managed individuals.

Table 2. Different letters indicate statistically significant differences at the $p \leq 0.05$ level, while identical letters denote non-significant differences.

Age groups (Year)	(Uric acid mg/dl) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	4.13±0.632 N=18 cd	3.17±0.436 N=16 fg	2.82±0.264 N=4 g
Group 2 (40-49)	4.46±0.461 N=17 bc	3.38±0.381 N=13 ef	3.24±0.541 N= 7 fg
Group 3 (50-59)	4.88±0.547 N=24 ab	3.83±0.240 N=5 de	3.70±0.436 N= 7 def
Group 4 (60-70)	5.13±0.569 N=11 a	4.05±0.247 N=4 cd	3.95±0.342 N=4 cd

4.4. Malondialdehyde (MDA)

4.4.1. Age-based comparisons of MDA levels:

A statistically significant increase ($p \leq 0.05$) in MDA levels was observed between the control group and Stage I thyroid cancer patients across all age categories.

A non-significant increase in MDA levels was detected between Stage I and Stage II patients across all age groups.

4.4.2. Across different age groups

Non-significant increases in MDA levels were observed within the control group, as well as among patients in Stage I and Stage II of thyroid cancer.

4.4.3. Interpretation and possible Causes

Malondialdehyde (MDA) is a key secondary product of lipid peroxidation and is one of the most widely used biochemical markers for evaluating oxidative stress. Elevated levels of MDA indicate cellular membrane damage due to the accumulation of free radicals (Al-Taee & Al-Helaly, 2024).

4.4.4. The possible reasons for elevated MDA levels in thyroid cancer patients include

• *Cancer-induced oxidative stress:* Numerous studies have demonstrated that tumors generate a pro-oxidative environment, leading to cellular damage and increased lipid peroxidation products such as MDA (Ayala *et al.*, 2014). Additionally, Aslan *et al.* (2011) reported significantly higher MDA levels in thyroid cancer patients compared to healthy individuals, emphasizing the role of oxidative damage in disease progression.

• *Post-surgical inflammatory response:* The inflammatory response following thyroidectomy may stimulate the release of free radicals, thereby enhancing lipid peroxidation and MDA production.

• *Hormonal deficiency following surgery:* Reduced levels of thyroid hormones after gland removal may disrupt metabolic homeostasis, adversely affecting redox balance in the body.

• *Weakened antioxidant defense system:* Decreased levels of antioxidants such as vitamin E and D, as indicated by previous results, may facilitate the accumulation of oxidative products like MDA (Halliwell & Gutteridge, 2015).

The findings also suggest that MDA levels tend to increase gradually with aging, even in healthy individuals. This trend is attributed to the enhanced generation of reactive oxygen species (ROS) with age, combined with a decline in the efficiency of natural antioxidants such as glutathione and catalase, leading to greater oxidative membrane damage and elevated MDA levels as an indicator (Del Rio *et al.*, 2005; Aziz, 2010).

Table 3. Different letters indicate statistically significant differences at the p ≤ 0.05 level, while identical letters denote non-significant differences.

Age groups (Year)	(MDA µmol/l) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	1.94±0.414 N=18 g	2.92±0.347 N=16 cd	3.36±0.234 N=4 bc
Group 2 (40-49)	2.11±0.345 N=17 g	3.28±0.433 N=13 cd	3.67±0.349 N= 7 bc
Group 3 (50-59)	2.28±0.408 N=24 fg	3.69±0.330 N=5 bc	3.85±0.231 N= 7 b
Group 4 (60-70)	2.54±0.543 N=11 ef	4.06±0.197 N=4 ab	4.31±0.419 N=4 ab

4.5. Glutathione

4.5.1. Age-based comparisons of GSH Levels

A significant decrease (p ≤ 0.05) in glutathione levels was observed between the control group and Stage I thyroid cancer patients across all age categories.

A significant decrease (p ≤ 0.05) in GSH was also observed between Stage I and Stage II patients in the fourth age group only, whereas the differences among other age groups were not statistically significant.

4.5.2. Differences across age groups

Non-significant reductions in GSH levels were noted within the control group and across both Stage I and Stage II patients, except for the significant decrease (p ≤ 0.05) observed between the first and second age groups in Stage II patients. This could be attributed to the transition from late youth (first group) to early middle age (second group), a stage marked by declining cellular regenerative capacity.

4.5.3. Interpretation and underlying mechanisms

The study findings revealed a consistent decline in glutathione (GSH) levels among all age groups of thyroid cancer patient's post-thyroidectomy, in comparison with the healthy control group. This persistent reduction suggests the presence of chronic oxidative stress that continues even after surgical removal of the malignant tissue.

Glutathione is one of the most critical intracellular antioxidants and represents the first line of defense against free radicals generated by metabolic activity or cancer-associated oxidative stress (Al Dleemy, 2009). According to Samarghandian *et al.* (2020), GSH serves as a sensitive marker for intracellular redox status, and its depletion often reflects impaired metabolic balance in cancerous cells.

A number of interconnected mechanisms may be responsible for decreasing the level of GSH in cancer patients, including:

Excessive ROS production inside the cancer cells, which leads to rapid depletion of GSH reserves (Traverso *et al.*, 2013).

Surgical inflammation, which contributes to oxidative stress in the nearby tissues (Estrela *et al.*, 2006).

Blocking the γ-glutamyl cycle in cancer cells directly disrupts GSH formation (Kaczmarek *et al.*, 2016).

Importantly, Stage II to Stage I differences in GSH levels were not consistently significant, in support of the hypothesis that GSH depletion is a precursor or early event in cancer development progression, and persists or does not persist regardless of whether clinical progression is evident. This suggests that GSH is a potential biomarker of carcinogenesis earlier rather than an indication of the severity of disease.

4.5.4. Age-Related Trends in GSH Levels: Presidency

On comparison of GSH levels across age groups in control, Stage I, and Stage II groups, progressive but non-significant decrease with advancing age was observed. This trend may be explained by:

Decrease in the efficacy of antioxidant defense mechanisms like glutathione with age.

Impaired mitochondrial activity and increased formation of ROS, thus greater consumption of GSH (Finkel & Holbrook, 2000).

Decreased ability for GSH regeneration caused by changes in metabolism that occur with age in bringing about the overall decrease in antioxidant.

Table 4. Different letters indicate statistically significant differences at the p ≤ 0.05 level, while identical letters denote non-significant differences.

Age groups (Year)	(GSH µmol/l) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	4.47±0.513 N=18 a	3.12±0.341 N=16 de	2.43±0.406 N=4 ef
Group 2 (40-49)	4.17±0.609 N=17 ab	2.84±0.269 N=13 eg	2.17±0.389 N= 7 gh
Group 3 (50-59)	3.86±0.729 N=24 bc	2.47±0.190 N=5 fg	1.95±0.139 N= 7 gh
Group 4 (60-70)	3.51±0.414 N=11 cd	2.20±0.215 N=4 g	1.64±0.369 N=4 h

4.6. Peroxynitrite

4.6.1. Age-based comparisons of peroxynitrite levels

A statistically significant increase (p ≤ 0.05) in $ONOO^-$ levels was observed between the control group and Stage I thyroid cancer patients across all age categories.

A statistically significant increase (p ≤ 0.05) was also recorded

between Stage I and Stage II patients in all age groups.

These results indicate a clear and significant elevation in peroxynitrite ($ONOO^-$) levels across all age groups in thyroid cancer patients—both in Stage I and Stage II—compared to the control group. Additionally, a significant incremental increase was observed between Stages I and II within each age category, suggesting that disease progression is accompanied by greater accumulation of reactive nitrogen species (RNS).

Peroxynitrite is considered one of the most cytotoxic byproducts of cellular stress and plays a central role in lipid peroxidation, protein oxidation, and DNA fragmentation in both cancerous and inflamed tissues.

The elevated $ONOO^-$ levels in thyroid cancer patients, even post-thyroidectomy, may be attributed to several interrelated physiological mechanisms:

Sustained production of reactive oxygen species (ROS) and nitric oxide (NO) by immune cells and residual tissue after surgery, leading to continued excessive formation of $ONOO^-$ (Pacher *et al.*, 2007).

Diminished detoxification capacity, primarily due to reduced levels of endogenous antioxidants such as glutathione (GSH) and vitamin E, which facilitates the accumulation of peroxynitrite (Traverso *et al.*, 2013).

Metabolic reprogramming in cancer cells, which disrupts the balance between ROS/RNS production and removal, allowing persistent nitrosative stress even after surgical intervention.

4.6.2. Age-related trends in peroxynitrite levels

Non-significant increases in $ONOO^-$ levels were observed among different age groups within the control group, Stage I, and Stage II patients, except:

In the control group, a significant increase ($p \leq 0.05$) was found between the first and second age groups, likely due to the shift from late youth to early middle age, a period marked by declining antioxidant efficiency.

In Stage II patients, a significant increase in $ONOO^-$ was observed between the third and fourth age groups, likely due to the combined effect of advancing age and disease severity.

These findings suggest that $ONOO^-$ levels tend to rise progressively with age in both healthy individuals and thyroid cancer patients. However, the increases were not consistently statistically significant. This trend is supported by studies indicating that aging is associated with a gradual rise in oxidative stress, caused by weakened antioxidant defense systems and diminished mitochondrial regulation of ROS production. These factors contribute to the age-dependent accumulation of harmful species like peroxynitrite (Dalle-Donne *et al.*, 2006; Finkel & Holbrook, 2000).

Table 5. Different letters indicate statistically significant differences at the $p \leq 0.05$ level, while identical letters denote non-significant differences.

Age groups (Year)	(ONOO µmol/l) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	21.75±2.318 N=18 i	35.32±3.133 N=16 f	39.66±2.390 N=4 de
Group 2 (40-49)	25.50±2.802 N=17 h	37.71±3.207 N=13 ef	42.49±2.743 N= 7 bcd
Group 3 (50-59)	27.90±4.343 N=24 gh	40.36±4.550 N=5 cde	44.92±2.846 N= 7 b
Group 4 (60-70)	29.52±3.545 N=11 g	43.81±4.917 N=4 bc	48.55±2.755 N=4 a

4.7. Vitamin E

4.7.1. Age-based differences in vitamin E concentration and its role in thyroid cancer

Within-group differences by disease stage

i. A statistically significant decrease ($p \leq 0.05$) in vitamin E concentration was observed between the control group and Stage I thyroid cancer patients across all age categories.

ii. A non-significant decrease in vitamin E concentration was noted between Stage I and Stage II thyroid cancer patients across all age categories.

4.7.2. Differences across age groups

A significant decrease in vitamin E concentration was recorded between age groups within the control group, while no significant differences were observed between age groups in Stage I or Stage II patients.

4.7.3. Biological significance and possible causes

Vitamin E is one of the most important lipid-soluble antioxidants and plays a critical role in protecting cellular membranes from oxidative damage. A decrease in vitamin E levels in cancer patients may indicate increased antioxidant consumption due to elevated oxidative stress associated with tumor progression or therapeutic interventions such as surgery, radioactive iodine therapy, or hormone replacement therapy(Thanoon *et al.*, 2010).

4.7.4. Potential causes of post-thyroidectomy vitamin E decline

i. Increased oxidative stress post-surgery: Surgical procedures and the accompanying inflammatory response lead to enhanced free radical production, which accelerates the consumption of antioxidants, including vitamin E (Gonzalez *et al.*, 2010).

ii. Reduced absorption or metabolic processing: Surgical and hormonal changes following thyroidectomy may impair the gastrointestinal absorption or metabolism of vitamin E, resulting in reduced serum levels.

iii. Cancer-driven antioxidant depletion: Studies suggest that cancer may cause an excessive consumption of antioxidants, including vitamin E, in an attempt to neutralize the excessive oxidative burden (Traber & Atkinson, 2007).

Several studies have confirmed that cancer patients generally present with markedly lower levels of vitamin E compared to healthy individuals (Yoshikawa *et al.*, 2000; Traber & Stevens, 2011).

Furthermore, the significant differences across age groups in the control group reveal an inverse relationship between age and vitamin E levels, which is attributed to decreased intestinal absorption, increased oxidative stress, and reduced efficiency in vitamin transport with aging and cellular senescence, as supported by Meydani (2005).

Table 6. Different letters indicate statistically significant differences at the $p \leq 0.05$ level, while identical letters indicate no significant difference.

Age groups (Year)	(Vitamin E mg/l) Mean ± SD		
	Control	Stage 1	Stage 2
Group 1 (30-39)	22.77±2.210 N=18 a	11.45±1.753 N=16 e	10.50±2.396 N=4 ef
Group 2 (40-49)	19.19±1.304 N=17 b	10.22±0.926 N=1 ef	9.96±0.906 N= 7 efg
Group 3 (50-59)	17.25±1.492 N=24 c	8.99±0.584 N=5 fgh	8.46±0.611 N= 7 gh
Group 4 (60-70)	15.57±1.483 N=11 d	7.84±0.277 N=4 h	7.38±0.557 N=4 h

Table 7. Determination of the Linear Correlation Coefficient Between Cyclin-Dependent Kinase 4 (CDK4) and Oxidative/ Antioxidative Factors in the Control Group

Clinical Variables	Controls N= 60
	R-value
-0.405**	Uric acid
0.438**	GSH
-0.432**	MDA
0.545**	Vit E
-0.464**	ONOO-

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

The results revealed a positive correlation between the activity of cyclin-dependent kinase 4 (CDK4) and the antioxidant markers glutathione (GSH) and vitamin E, suggesting that an antioxidant-rich intracellular environment may support the proper regulation of the cell cycle. In contrast, moderate negative correlations were observed between CDK4 and the oxidative stress markers malondialdehyde (MDA), peroxynitrite ($ONOO^-$), and uric acid, indicating that elevated oxidative stress is associated with reduced CDK4 activity.

These findings indicate that CDK4 is sensitive to the redox status within cells, even under physiological conditions, highlighting the critical role of oxidative balance in regulating cell cycle progression.

Table 8. Determination of the Linear Correlation Coefficient Between Cyclin-Dependent Kinase 4 (CDK4) and Oxidative/ Antioxidative Factors in Thyroid Cancer Patients

Clinical Variables	Patients N= 60
	r-value
Uric acid	-0.437**
GSH	0.078
MDA	-0.262*
Vit E	0.310*
ONOO-	-0.072-

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

Post-thyroidectomy results indicated a significant redox-dependent influence on CDK4 activity. High negative correlations between CDK4 and uric acid, and malondialdehyde (MDA), and weak positive correlation with vitamin E were observed. However, the correlation with glutathione (GSH) and peroxynitrite ($ONOO^-$) was not statistically significant, which suggests a disrupted oxidative balance or regulatory alteration following surgery. These findings indicate that CDK4 remains sensitive to biochemical heterogeneity despite tumor resection, making it potentially valuable for assessing cellular stability in the postoperative period.

5. CONCLUSION

This study identifies cyclin-dependent kinase 4 (CDK4) as a sensitive cellular status marker for thyroid cancer. CDK4 levels were significantly elevated in patients—and significantly as the disease advanced—highlighting CDK4's role in amplifying malignant cell proliferation through activation of the CDK4/ Cyclin D pathway and suppression of cell-cycle inhibitors like p16^INK4a. Most intriguingly, tumor-induced up-regulation of CDK4 overcame age-related physiological effects, as patient age had no significant effect on enzyme activity.

In parallel, a pronounced redox unbalance persisted following thyroidectomy: oxidative indicators (MDA, $ONOO^-$) were increased to a great extent, while antioxidant protection (GSH, vitamin E) was reduced. This confirms the thesis of ongoing oxidative stress—possibly due to microscopic disease residue or post-surgical inflammatory responses.

These results were confirmed by correlative analysis, which revealed negative correlations between CDK4 and uric acid and MDA, and a positive correlation with vitamin E in patients, indicating that chronic oxidative stress still impacts cell-cycle dynamics and may contribute to the aggressiveness of tumors.

In combination, the results illustrate CDK4's dual value as an early marker and a target for therapy. They also illustrate the significance of monitoring and maintaining oxidant status as part of thyroid cancer postoperative management.

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