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Thyroid-pineal axis: Melatonin, circadian clock gene expression and vitamin D in Hashimoto's thyroiditis patients.

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ABSTRACT:

Background: There is evidence to suggest that thyroid hormone may affect circadian rhythm.

Aim: Investigate changes in peripheral blood expression of circadian clock genes and melatonin and vitamin D in Egyptian women with Hashimoto Thyroiditis (HT).

Methods: Case–control study included 240 females divided into two groups: **(Control group):** 120 euthyroid healthy females & **(HT group):** 120 females with HT. Serum thyroid hormones, antibodies, melatonin, (25(OH)D), ionized calcium, hemoglobin were measured, Determination of gene expression of the brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (*BMAL-1*), Period 2 (*PER2*), nuclear factor kappa B (*NF-κB*), sirtuin 1 (*SIRT1*), Toll-like receptor 4 (*TLR4),* and tumor necrosis factor alpha (*TNF-α*) , which are associated with the circadian clock.

Results: Melatonin, vitamin D, ionized calcium, and hemoglobin were lower in the HT group. Expression of *BMAL-1*, *PER2*, and *SIRT1* was downregulated , whereas that of *NFKB*, *TLR4*, and *TNF*-α was upregulated in the HT group.

Conclusion: Suggests associations between HT and circadian rhythm, as reflected in the downregulation of *BMAL-1* and *PER2* gene expression, inflammatory response, and oxidative stress. The significant decrease in serum melatonin levels in HT patients strongly suggests an association between melatonin pathway and HT.

Keywords: Hashimoto's thyroiditis, Circadian rhythms, Melatonin, Vitamin D, Circadian clock genes, Thyroid –pineal axis

1. INTRODUCTION

Hashimoto's thyroiditis (HT) is an autoimmune endocrine disease characterized by the presence of antibodies to thyroid peroxidase (TPO) and thyroglobulin (TG). Thyroid tissue is attacked by these antibodies, leading to insufficient thyroid hormone production (Antonini et al., 2023). The disease occurs 4-10 times more frequently in females than males (Ihnatowicz et al., 2021; Pyzik et al. 2015). The thyroid gland plays a vital role in regulating metabolism and energy levels by producing thyroid hormones (T3 and T4). It may have an impact on the body's circadian rhythm, which is the internal 24-hour clock that regulates sleep-wake cycles and various physiological processes. Although there is no direct research linking Hashimoto's thyroiditis and circadian rhythm disturbances, evidence suggests that thyroid hormone may affect the circadian rhythm and seasonal rhythmicity (Shahid et al., 2025).

The circadian clock consists of interlocked transcriptional-translational feedback loops (Lin et al., 2024). During the daytime, the circadian locomotor production cycle kaput (CLOCK) and brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (*BMAL-1*) transcription factors heterodimerize and bind to elements that regulate expression of the period circadian regulator genes (*PER2*, and *PER3)* and the Cryptochrome (*CRY1* and *CRY2*) circadian regulator genes (Reilly et al., 2007). For a long period of time, it was thought that neurons of the suprachiasmatic nucleus (SCN) uniquely controlled the circadian rhythmicity of peripheral tissues via neural and humoral signals. However, it is now accepted that peripheral tissues like the cardiovascular system have a circadian clock similar to that in the SCN (Reilly et al., 2007). This has raised the possibility that biological responses under the control of the molecular clock might interact with environmental cues like changes in hormonal status to influence the patient's health.

In mammals, it is well documented that observable circadian rhythms are controlled by a central oscillator that is organized in transcriptional and translational feedback loops involving several clock genes. The demonstration of functional circadian machinery in human PBMCs suggests that peripheral

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blood cells may be useful for the investigation of human circadian rhythms and their associated disorders. As PBMCs have been widely used for more than 10 years now as a valuable tool to provide reliable biomarkers of health and diseases (Boivin et al., 2003).

The regulators of circadian clock genes include mainly melatonin, Sirtuin1, and vitamin D. Melatonin is a hormone that plays a crucial role in the regulation of circadian rhythm produced by the pineal gland in the brain in response to darkness and is suppressed by bright light (Cajochen et al., 2003). One of the key functions of melatonin is to promote sleepiness and regulate the timing and duration of sleep (Aulinas, 2019). Melatonin levels rise in the evening as darkness falls, signaling the body to prepare for sleep. Melatonin receptors in the brain mediate this effect (Cajochen et al., 2003). In addition to its sleep-promoting effects, melatonin also helps to regulate body temperature, blood pressure, and hormone secretion. These processes are all synchronized by the body's internal clock, which is regulated by melatonin and other circadian factors (Masters et al., 2014).

Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase that plays a crucial role in various physiological processes, including the regulation of circadian rhythm. *SIRT1* regulates the activity of the core clock genes, including *CLOCK, BMAL1, PER, and CRY*, by interaction with these core clock genes and regulates their acetylation status, thereby modulating their transcriptional activity and stability (Asher et al., 2008).

Vitamin D is a fat-soluble hormone that plays a vital function in the homeostasis of calcium levels, skeletal growth, and skeletal maintenance. Vitamin D and thyroid hormones bind to the same steroid hormone receptors. There have been studies of a different gene in the vitamin D receptor predisposing individuals to autoimmune thyroid disease, including Graves' disease (Botelho et al., 2018; Qu et al., 2017). Besides these metabolic effects, Vitamin D has an important regulatory role in controlling the circadian clock genes, including *BMAL1* and *PER2* (Gutierrez-Monreal et al., 2014).

There are many disruptors of the circadian rhythm, including the proinflammatory mediatorsand the toll-like receptor (TLR) pathways. Activation of TLR leads to an innate immune response and the production of mediators of inflammation such as TNF-α and interleukin (6, 12, and 18). TLRs also stimulate nuclear factor-kappa B (NF-κB), which translocates into the nucleus and enhances the expression of proinflammatory genes. NF-κB is also involved in normal thyroid development and function and is implicated in the expression of several genes unique to the thyroid, such as TPO and TG (Giuliani et al., 2018; Reale et al., 2018). It was demonstrated that TLR-4 signaling activation disrupted the circadian rhythm of clock genes, subsequently affecting the immune response (Baxter & Ray, 2020).

The possible crosstalk between Hashimoto's thyroiditis and the peripheral circadian clock genes and their regulators has not yet been investigated. So, the present study aimed to investigate the changes in the peripheral blood expression of circadian clock genes *BMAL1* and *PER2, SIRT1, NF-κB*, and *TNF-α*, and the circulatory levels of melatonin and vitamin D in Egyptian women with Hashimoto's thyroiditis. We aimed to a spotlight a new axis in endocrinology (Thyroid-Pineal axis) via a study of the possible association between the melatonin pathway and HT.

1. METHODS

2.1 Participants

Ethics approval for the study was obtained from the Medical Research Institute Ethical Committee, University of Alexandria, Alexandria Governorate, Egypt. Written informed consent was obtained from all participants included in the study. The research was conducted in accordance with the World Medical Association's Code of Ethics (Declaration of Helsinki). Protocol serial number: E/C. S/N. T56/2019.

This case–control prospective study was conducted during 1 year period from January 2019 to December 2019. The participants were selected by convenient sampling from the general population of females (aged 20-45 years). The participants were divided into two groups: **Group I** (**Control group**): 120 euthyroid healthy females aged 20-45 years with a mean ± SD of 35±11.56 years, without clinical evidence or a family history of any autoimmune diseases, were selected to participate in this study after recording histories, physical examination and confirmation of the normal FT3, FT4, TSH, and negative thyroid autoantibodies. **Group II (HT group**) consisted of 120 females with newly diagnosed untreated Hashimoto's thyroiditis. They were recruited via a health center before being treated and matched in age with group I. All the participants were Egyptian. The diagnosis of Hashimoto's thyroiditis was made when patients present with symptoms of hypothyroidism, accompanied by laboratory testing of hypothyroidism, which is an elevated TSH with or without low FT4 and FT3 levels. Typical diagnosis of Hashimoto's thyroiditis was made by detecting elevated serum levels of anti-TPO (TPO-Ab), and anti-thyroglobulin antibodies (Tg-Ab) (Liu et al., 2016).

The exclusion criteria were: history of endocrinopathies of the immune system other than HT, prior or current use of medications known to impair thyroid functions and/or thyroid immunity (interferon or glucocorticoids), diabetes, malignancy, and use of melatonin supplements.

Venous blood was withdrawn from all participants at 9 AM and collected into two tubes. The first tube collected into vacuum tubes containing EDTA as an anticoagulant to assess *BMAL-1, PER2, TNF-α, SIRT-1, NF-κB, and TLR-4* gene expressions, and hemoglobin concentration. The second tube was then put into the serum vacutainer, allowed to clot for 30 min, and then centrifuged at 4,000 rpm for 10 minutes at room temperature to obtain serum for assessment of Melatonin, Vitamin D, thyroid stimulating hormone (TSH), Free T4, Free T3, Anti-TPO, Anti-TG, and Ionized calcium

2.2 Methods

2.2.1 Determination of serum TSH (Sarkar, 2014), FT3, FT4 (Kazerouni & Amirrasouli, 2012) levels

TSH, FT3, and FT4 were determined using an electrochemiluminescence immunoassay on Roche Elecsys immunoassay analyzers.

2.2.2 Determination of serum anti-TPO-Abs and anti-Tg-Abs levels (Intan et al., 2014)

Anti-TPO-Abs and anti-Tg-Abs levels were determined using an electrochemiluminescence immunoassay on Roche.

2.2.3 Determination of serum ionized calcium (Ca^{+2}) (Burnett et al., 2000)

An automated ion-selective electrode analyzer measured serum-ionized calcium.

2.2.4 Determination of blood hemoglobin concentration (Hb) (Grillone et al., 2014)

Hb level was measured using a Sysmex count full automation hematology analyzer.

2.2.5 Determination of serum melatonin (Milon et al., 2019)

Melatonin was determined using ELISA kits purchased from Bioneovan Co., Ltd. and absorbance was measured at 450 nm.

2.2.6 Determination of serum vitamin D (Saida et al., 2018)

Vitamin D3 was determined by immunoturbidimetric kits purchased from spectrum Company for Biotechnology.

2.2.7 Gene expression of *BMAL-1, PER2, TNFα, SIRT-1, NF-κB,* and *TLR-4* by RT-PCR.

The total RNA was collected from peripheral blood samples using the TRIzol RNA Isolation Technique (Invitrogen). Reverse transcriptase-polymerase chain reaction (RT-PCR) cDNA synthesis. The HiSenScript™ RH (-) cDNA Synthesis kit was purchased from iNtRON Biotechnology, Inc, in compliance with orders from the manufacturer. Relative quantification of gene expression was performed using the SYBR Green PCR Kit, which is a convenient premix of the components (except primers, template, and water) needed to perform real-time PCR using SYBR® Green I Dye. By calculating the increase in fluorescence caused by the binding of SYBR® Green dye to double-stranded (ds) DNA, direct detection of the PCR product is controlled (Gowayed et al., 2020). The Beta actin (ACTB) housekeeping gene was used as a reference gene for normalization. Primers used for genes were as follows:

Primers sequences

Gene	**Primer sequence**	
TLR-4	F:	5'- CACCTGTAGTGCTGTGTCGTT -3'
	R:	5'- TCACATCTGAGGGCACCTAAG -3'
SIRT-1	F:	5'- AGAGCCTCACATGCAAGCTCTAG -3'
	R:	5'- GCCAATCATAAGATGTTGCTGAAC -3'
NF-κB	F:	5'- GAGACATCCTTCCGCAAACT-3'
	R:	5'- GTCCTTCCTGCCCATAATCA-3'
TNF-α	F:	5'- CAGAGGGCCTGTACCTCATC-3'
	R:	5'-GGAAGACCCCTCCCAGATAG-3'
Bmal-1	F:	5'- CACTGGAAGGAATGTCTGG-3'
	R:	5'-GGAAGACCCCTCCCAGATAG-3'
PER2	F:	5'- AGCCAATGAAGAGTATTACCAG-3'
	R:	5'- GCCACCGCAAACATATCG-3'
ACTB	F:	5'-CACCATTGGCAATGAGCGGTTC-3'
	R:	5'-AGGTCTTTGCGGATGTCCACGT-3'

Rotor-Gene Q-Pure Detection Version 2.1.0 (build 9) (Qiagen®, Valencia, CA, USA) has determined the threshold period (Ct) values. mRNA relative change in samples was calculated for each gene using the $2^{-\Delta\Delta Ct}$ method and normalized to the ACTB housekeeping gene.

2.2.10 Statistical Analysis

The data was analyzed using version 18.0 of the SPSS software package (SPSS Chicago, IL, USA). The data were expressed mean ± SD and analyzed using an independent t-test to compare between two different groups and Pearson for correlation study. The P-value was assumed to be significant at $p \leq 0.05$.

3. Results

3.1 Thyroid functional hormones, Antibody to TPO & TG

Hashimoto's thyroiditis group showed significantly higher TSH levels and antibodies to TPO and TG concentrations than the control group. In contrast, FT_3 and FT_4 levels in the HT group were significantly lower than in the control group (**Table 1**).

3.2 Ionized Calcium and Hemoglobin Concentrations

Hashimoto's thyroiditis group showed significantly lower serum Ca^{+2} and Hb concentrations than the control group; the percent difference were (-11.1%, -24.2%) respectively. **(Table 1)**

3.3 Serum Vitamin D (ng/mL)

In the HT group, serum vitamin D concentrations were significantly lower than in the control group; the percent difference was -30.7%. **(Table 1)**

3.4 Serum Melatonin (pg/mL)

Hashimoto's thyroiditis group had a significantly lower serum melatonin concentration than the control group; the percent difference was -48.6% **(Table 1).**

Table (1): Laboratory findings in the control group and HT group

Variable	Control Group (n = 120)	HT Group (n = 120)	Figure	P
TSH (µIU/mL)	2.6 ± 0.66	34.7 ± 6.2 *	1225.2 %	<0.001*
Free T_3 (pg/mL)	2.5 ± 0.40	1.6 ± 0.56 *	-33.1%	<0.001*
Free T_4 (ng/dL)	1.5 ± 0.25	0.8 ± 0.15 *	-44.1%	<0.001*
Antibody to TPO (IU/mL)	11.4 ± 2.5	229 ± 34.4 *	1901.1%	<0.001*
Antibody to TG (IU/mL)	15.6 ± 3.02	324.4 ± 73.7 *	1975.5%	<0.001*
Ionized calcium (mg/dL)	5 ± 0.17	4.4± 0.22 *	-11.1%	<0.001*
Hemoglobin (g/dL)	13.3 ± 0.62	10.1 ± 0.56 *	-24.2%	<0.001*
Vitamin D (25(OH)D) (ng/mL)	22.80 ± 5.46	15.03 ± 7.29	-30.7%	<0.001*
Serum Melatonin (pg/mL)	33.22 ± 8.45	17.03 ± 4.11	-48.6%	<0.001*

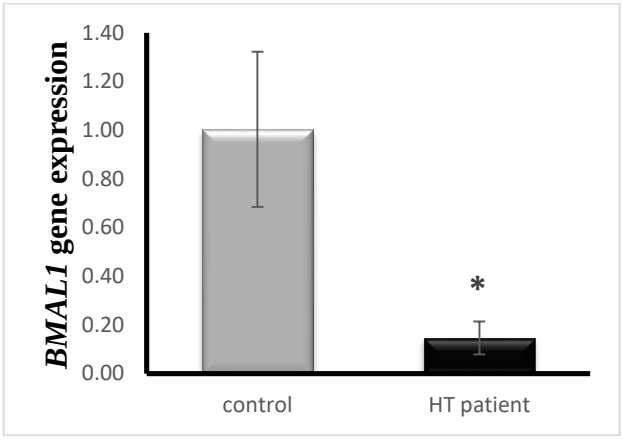
Data presented as mean ± SD. (n=120)

*: Significantly different from the control group by independent t-test (p≤0.05)

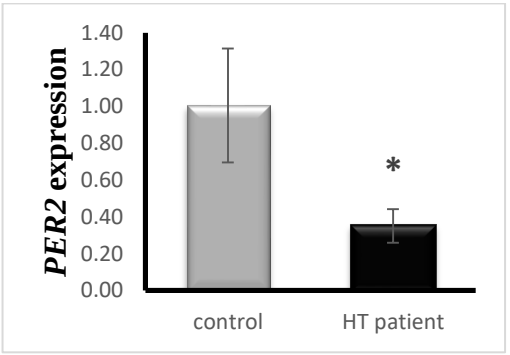
Abbreviations: FT3, free triiodothyronine; FT4, free thyroxine; HT, Hashimoto's thyroiditis.

3.5 Gene expression of *BMAL1, PER2, SIRT1* (fold change)

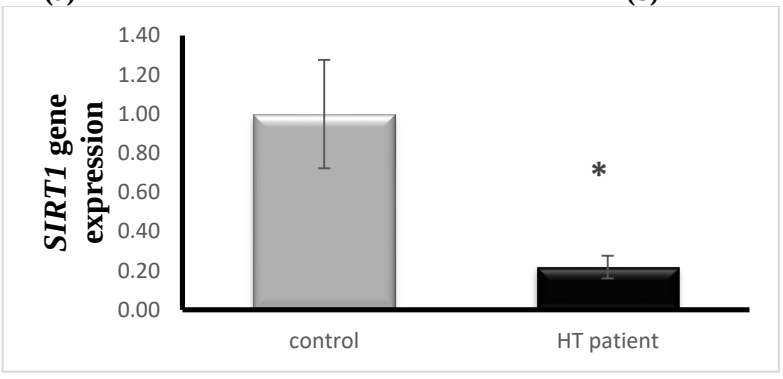
Hashimoto's thyroiditis group showed a statistically significant down regulation in *BMALI*, *PER2*, and S*IRT1* expressions compared to the control group; percent differences were (-85%, -65.1%, -78.1%) respectively **(Fig. 1 a,b,c).**



(a)



(b)



(c)

Fig. (1): (a) *BMAL1* expression in the control and HT groups. (P<0.001*), (b) *PER2* expression in the control group and HT group. (P<0.001*), (c) *SIRT1* expression in the control group and HT group. (P<0.001*)

3.6 Gene expression of *TNFα, NF-κB, TLR4* (Fold change)

Hashimoto's thyroiditis group showed a statistically significant (3, 6, 7 fold) upregulation in *TNFα, NF-κB, and TLR4* expression compared to the control group respectively; percent differences were (101.8%, 404.2%, 547.9%) respectively (**Fig. 2 a,b,c).**

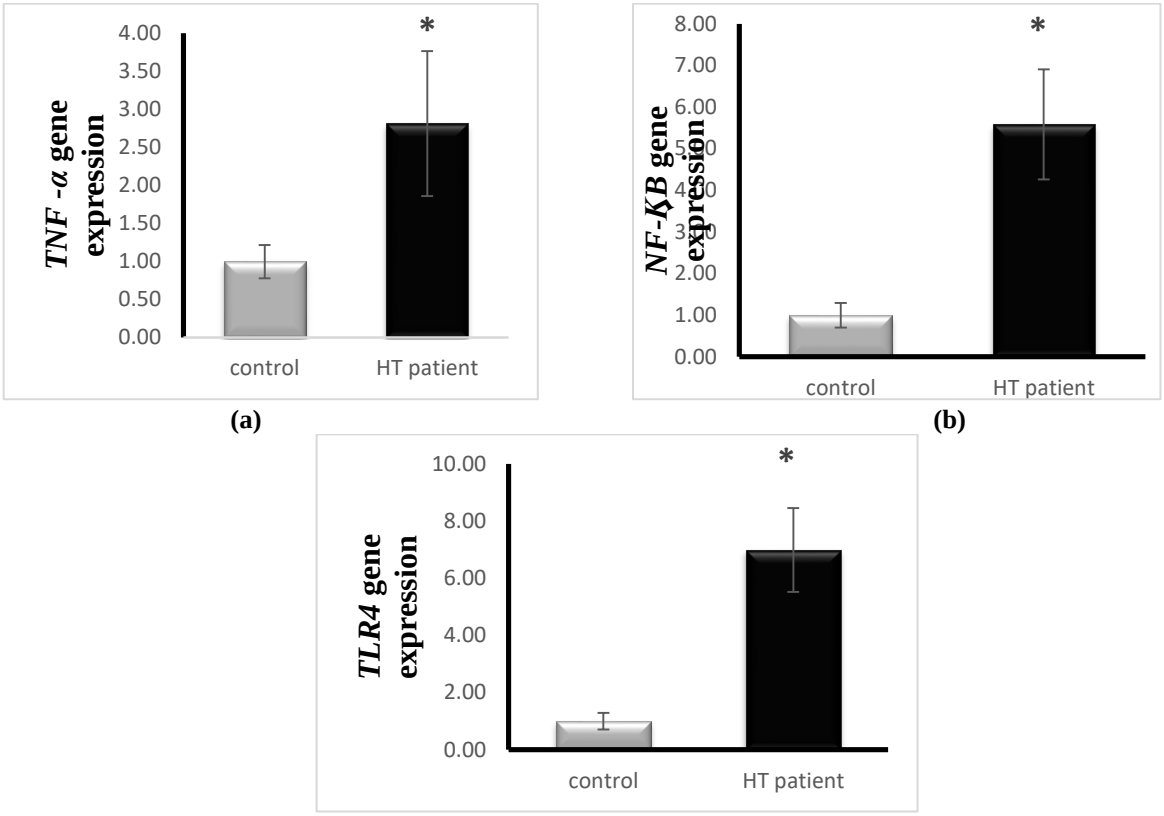
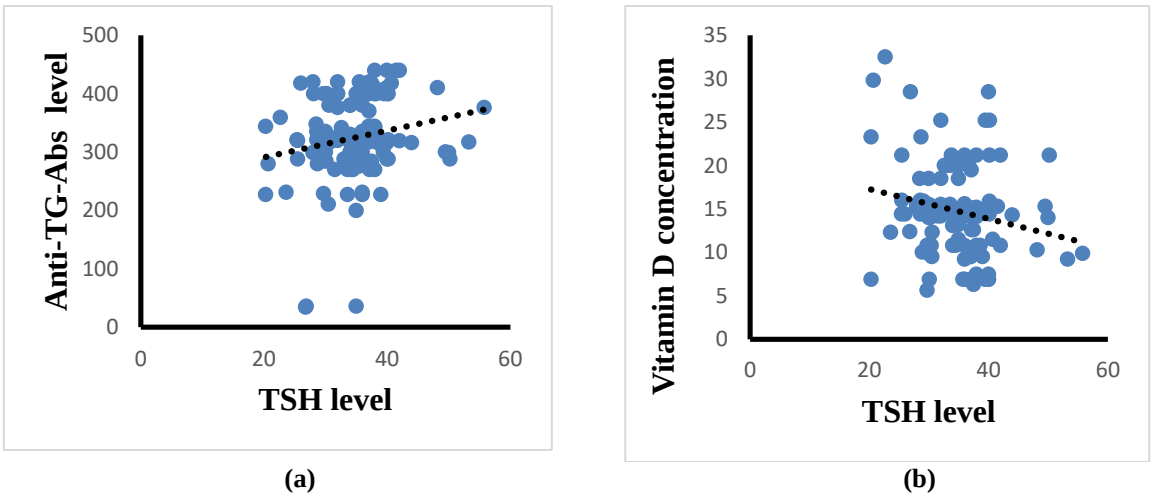
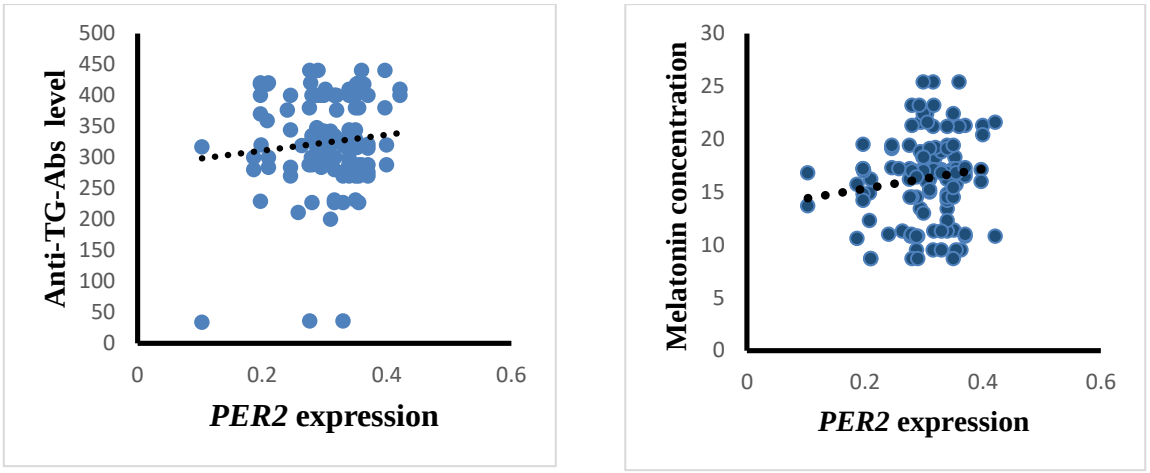


Fig. (2): (a) *TNFα* expression in the control and HT groups. (P<0.001*), (b): *NF-κB* expression in the control group and HT group. (P<0.001*), (c): *TLR4* expression in the control group and HT group. (P<0.001*)

3.7 Correlation studies

The statistical analysis using Pearson correlation reveals that TSH level is directly correlated with anti-TG-Abs level (r=0.446, p=0.014, **Fig. 3a**) and inversely correlated with vitamin D concentration (r=-0.442, p=0.015, (**Fig. 3b**) in HT group. *PER2* expression is directly correlated with anti-TG-Abs level (r=0.435, p=0.016, (**Fig. 3c**) and melatonin concentration (r=0.506, p=0.004, (**Fig. 3d**) in the HT group.





(c) **(d)**

Fig. (3): Correlation curve between TSH level and anti-TG-Abs (a), TSH level and vitamin D concentration (b), *PER2* expression and anti-TG-Abs, and (c) *PER2* expression and melatonin concentration (d) in HT group

4. Discussion

Hashimoto thyroiditis (HT) is a chronic autoimmune thyroiditis in which thyroid cells are destroyed by antibodies to TPO and TG (Milo et al., 2023). Our findings of increased concentrations of these antibodies in the HT group and reduced levels of FT_3 and FT_4 are consistent with previous studies. According to our findings, Barić et al. found a significant increase in antibodies to TPO and TG and TSH levels in individuals with HT accompanied by a significant decrease in FT_3 and FT_4 (Barić et al., 2019). Our study showed a significant direct correlation between levels of TSH and increased concentrations of antibodies to TG.

Thyrocytes are strictly epithelial cells that produce the thyroid hormones (T_3 and T_4) (Tipu et al., 2018). Cytokines produced by T-cells lead to the expression of HLA-DR surface antigens on thyrocytes, rendering them vulnerable to immune attack. Polyclonal antibodies are directed against the same epitopes in healthy individuals and patients. In healthy individuals, antibodies to TPO did not block TPO activity. Patients with autoimmune thyroid diseases interfere with the antibody-blocking activity while activating complement, which destroys thyrocytes and acts as a competitive enzyme activity inhibitor (Fröhlich & Wahl, 2019; McLachlan & Rapoport, 2004).

In the present study, low hemoglobin levels were observed in participants with HT, which is consistent with the low hemoglobin levels and anemia reported in patients with hypothyroidism (Ahmed & Mohammed, 2020; Salem et al., 2021). Iron is vital for the activity of TPO. Many studies have shown that iron deficiency reduces the activity of TPO and can also lead to depression of thyroid function. Important negative associations between TSH and hemoglobin levels have been observed (Khatiwada et al., 2016). There is also an association between anemia, thyroid function, and metabolic status; thyroid hormones stimulate the proliferation of erythrocyte precursors and promote erythropoiesis by increasing the expression of erythropoietin gene and the development of erythropoietin in the kidneys (Kim et al., 2020).

Our finding of the low ionized calcium levels in the HT group is in accordance with several previous studies (Mani et al., 2019; Susanna et al., 2016). Vitamin D is one of the main hormones that regulate calcium levels and other minerals in blood; it promotes the calcium absorption from the gut and influences calcium and phosphorous bone turnover and renal excretion (Ahi et al., 2020). Considering that we found reduced vitamin D levels in the HT group. This deficiency could have led to decreased concentrations of serum-ionized calcium.

The association between vitamin D deficiency and thyroid autoimmunity has been demonstrated in several studies. For example, cholecalciferol supplements successfully reduce antibodies to TPO in patients with HT because they have anti-inflammatory and immunomodulatory functions (Liontiris & Mazokopakis, 2017). Our study showed a significant inverse correlation between TSH and vitamin D levels. A recent study by Salem et al. in 2021 reported the association between autoimmune thyroiditis, iron deficiency anemia, and vitamin D deficiency. The authors grouped the three parameters (Thyroiditis, iron & vitamin D) in one new association called TAD association syndrome (Salem et al., 2021). The relationship between TAD association syndrome and the pineal gland is an area of active investigation.

Hypothyroidism can affect peripheral circadian clocks (Peliciari-Garcia et al., 2018). As shown in our study, there was a downregulation of *BMAL1* and *PER2* genes in the HT group. Perturbation of the circadian system leads to disturbance of thyroid function and cell-cycle progression (Ikegami et al., 2019). One of the significant mechanisms thought to mediate the effects causing circadian misalignment is the generalized disruption of the endocrine system. TSH and thyroid hormones display seasonal variations in the blood (Kuzmenko et al., 2021).

There are many disruptors of the circadian rhythm, including the proinflammatory mediators and the toll-like receptor (TLR) pathways. Activation of TLR leads to an innate immune response and the production of mediators of

inflammation such as TNF-α and nuclear factor-kappa B (NF-κB) (Zeng et al., 2024).

TNF-α interferes with circadian clock control and induces extended rest periods in the dark by suppressing the expression of period circadian regulator genes through activating p38 mitogen-activated protein kinases. This process is associated with the development of autoimmune diseases (Ertosun et al., 2019).

TNF-α is a cytokine produced at inflammatory sites by activated macrophages and monocytes and is produced by thyroid epithelial cells in patients with autoimmune disease (Zhu et al., 2020). Tumor necrosis factor-alpha is known to modulate the expression of thyroid-specific genes, contributing to the pathogenesis of autoimmune thyroid diseases. TNF-α might play a role in the regulation of the pituitary–thyroid axis (Díez et al., 2002). Aust et al. found high levels of *TNF-α* mRNA in tissues from patients with Hashimoto's thyroiditis (Aust et al., 1996). Pang et al. (Pang et al., 1989) have demonstrated TNF receptors on human thyroid cells, whichcould play a role in autoimmune thyroid disease (Tandon et al., 1994). Also, TNF-α has been implicated in the low T3. It might inhibit the peripheral generation of T3 from T4 and act as an autocrine factor by decreasing thyroidal generation and release of T3 (Ongphiphadhanakul et al., 1994). An increase in serum levels of TNF-α has been found in patients with autoimmune thyroiditis (Drugarin et al., 2000). As TNF-α is involved in the regulation of thyroid growth and function, serum T3 and T4 levels were reduced in mice or rats on administration of TNF-α (Ohmori et al., 1999). This effect supports our finding of increased *TNF-α* expression in participants with HT.

In mammals, NF-κB can alter the circadian clock by interaction with BMAL-1 protein and by modification of circadian rhythms in suprachiasmatic nucleus activity. Shen et al. found that NF-κB transcription factor p65, like CRY1, can suppress *BMAL1–hCLOCK* operation at E-box elements and compete with coactivator CBP/p300 to bid for *BMAL1–hCLOCK* binding. Chromatin immunoprecipitation revealed that p65 and *BMAL1–hCLOCK* binding sites collect on the E-box elements of circadian genes. These findings support the vital function of NF-κB in the control of the circadian system and the connection between inflammation and circadian pathways (Shen et al., 2021). We found significant upregulation of *NF-κB* in our HT group participants.

We also found that *NF-κB and TLR4* expression in peripheral leukocytes was significantly upregulated in the HT group compared with the control group. Previous studies found higher serum concentrations of HSP-60 and HMG-1 (both ligands of TLR-4) in HT patients than in healthy controls. Ligand binding to TLR-4 leads to the activation of the TLR-4/MyD88-dependent pathway. A positive correlation between HSP-60 and autoantibodies against TPO and TG in HT patients has been suggested (Mardente et al., 2010; Peng et al., 2016). NF-κB activation occurs via cell membrane receptors, such as TLR-4, which can induce cell proliferation, inflammatory response, and immune system cell recruitment for cancer progression (Chuffa et al., 2016).

Faria et al. (Faria et al., 2020). proposed that the canonical NF-κB pathway may be involved in sodium iodide symporter downregulation. *NF-κB* provides a mechanism by which immune activation can lead to dysregulation of the circadian rhythm. Upregulation of *NF-κB* leads to inhibition of clock repressor genes, leading to the disruption of clock cycles and alteration of the circadian rhythm in mice (Hong et al., 2018). *NF-κB* can also relocalize the *hCLOCK–BMAL-1* complex at new sites in the genome, close to sites bound by *NF-κB*, to regulate transcription following inflammatory stimuli (Hong et al., 2018).

The regulators of circadian clock genes include mainly melatonin and Sirtuin1. *hSIRT1* binds to *hCLOCK* and is introduced by circadian gene promoters to *the hCLOCK–BMAL1* heterodimer. *hSIRT1* deacetylates H3K9 and H3K14 at these sites, as well as the *BMAL1* and *hPER2* non-histone proteins. Inhibition or deletion of *SIRT1* leads to changes in the circadian cycle (Nakahata et al., 2008), which was indicated in the current study by the reduced expression of *PER2* and *BMAL1*.

Of the seven sirtuin variants, *hSIRT1* has the strongest deacetylase activity. There is considerable evidence that sirtuins react through direct deacetylation of transcription factors to control antioxidant gene expression in reaction to oxidative stress. Indeed, oxidative stress can be attenuated by *hSIRT1*, and downregulation of *hSIRT1* has been associated with increased systemic oxidative stress (Al-Khaldi & Sultan, 2019), which is compatible with our findings in HT patients.

Melatonin is produced predominantly in the pineal gland, and its synthesis and release depend on circadian rhythms (Tan et al., 2010). We found reduced levels of melatonin in the serum of participants in the HT group. In the pineal gland, NF-κB activation through TLR-4 leads to translocation of p50–p50 and p50–p65 dimers into the nucleus. Nuclear transport of these dimers decreases noradrenaline-induced arylalkylamine-*N*-acetyltransferase (AA-NAT) mRNA transcription, leading to reduced melatonin synthesis. The BMAL-1 circadian clock protein plays a key role in circadian rhythms and regulates the regular rhythms of melatonin (Vriend & Reiter, 2015).

AA-NAT is the rate-limiting enzyme in the synthesis of melatonin from L-tryptophan. Via the E-box in its promoter, BMAL1–hCLOCK heterodimers also transactivate AA-NAT gene transcription (Chen & Baler, 2000). The present study showed that PER2 expression directly correlated with levels of antibodies against TG and melatonin.

Increased melatonin concentrations suppress the expression of the *NF-κB p65* subunit, whereas expression of the *NF-κB* inhibitor epsilon (IkB-E) protein is increased. It can also inhibit the expression of *TLR-4* and decrease the levels of MyD88 in the cytosol, thus blocking the induction of *NF-κB*. Melatonin may also prevent the phosphorylation of IkB-E by I-kappa-B kinase alpha, which prevents *NF-κB* activation and binding to multiple genes in the promoter region (Ordoñez et al., 2014).

5. Conclusion

Our findings showed an association between HT and circadian rhythms, as reflected in the downregulation of *BMAL-1* and *PER2* gene expression compared with health controls. Moreover, this association may lead to an inflammatory response and oxidative stress. The significant decrease in serum melatonin levels in HT patients strongly suggests an association between the melatonin pathway and HT.

However, the small sample biased recruitment and absence of a longitudinal design are a major limitation of the present study. Extra studies are needed for understanding the pathogenesis of Hashimoto thyroiditis. Melatonin can be used as supplementation to compensate for the effect of Hashimoto thyroiditis. Further studies on the circadian genes and circadian rhythm in thyroid function are required.

Statements and declarations

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There are no conflicts to declare

Competing Interests

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Author Contributions

Sara. A. Shaker: Supervised the practical part, data analysis & interpretation and writing of the manuscript.

Magda A. Megahed: Proposed research plan, reviewed manuscript and accepted it, analyzed and interpreted the result.

Mahmoud Mahfouz: Performed practical work, reviewed and accepted manuscript.

Tamer E. Hassanien: Performed practical work, reviewed and accepted manuscript.

Bothaina F Mahmoud: Supervised the practical part and analyzed the results and interpretation, reviewed the manuscript and accepted it.

Tarek M. Salem: Concept and design, recruiting patients, acquisition of data, data analysis & interpretation and critical revision of the manuscript.

Data Availability

The data generated and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Ethics approval

Approval is taken by Medical Research Institute Ethical Committee, University of Alexandria, Alexandria, Egypt. The research was performed for studies involving humans according to the World Medical Association's Code of Ethics (Declaration of Helsinki).

Consent to participate

All the participants in the study signed an informed written consent before participating in the research.

Consent to publish

Not applicable

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References

Ahi, S., Dehdar, M. R., & Hatami, N. (2020). Vitamin D deficiency in non-autoimmune hypothyroidism: a case-control study. *BMC Endocr Disord, 20*(1), 41.

Ahmed, S. S., & Mohammed, A. A. (2020). Effects of thyroid dysfunction on hematological parameters: Case controlled study. *Ann Med Surg (Lond), 57*, 52-55.

Al-Khaldi, A., & Sultan, S. (2019). The expression of sirtuins, superoxide dismutase, and lipid peroxidation status in peripheral blood from patients with diabetes and hypothyroidism. *BMC Endocr Disord, 19*(1), 19.

Antonini, S., Birtolo, M. F., Lania, A., & Longhi, E. V. (2023). Hashimoto Thyroiditis *Managing Psychosexual Consequences in Chronic Diseases* (pp. 95-102). Cham: Springer International Publishing.

Asher, G., Gatfield, D., Stratmann, M., Reinke, H., Dibner, C., Kreppel, F., . . . & Schibler, U. (2008). SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell, 134*(2), 317-328.

Aulinas, A. (2019). Physiology of the pineal gland and melatonin.

Aust, G., Heuer, M., Laue, S., Lehmann, I., Hofmann, A., Heldin, N. E., & Scherbaum, W. A. (1996). Expression of tumour necrosis factor-alpha (TNF-alpha) mRNA and protein in pathological thyroid tissue and carcinoma cell lines. *Clin Exp Immunol, 105*(1), 148-154.

Barić, A., Brčić, L., Gračan, S., Škrabić, V., Brekalo, M., Šimunac, M., . . . & Boraska Perica, V. (2019). Thyroglobulin Antibodies are Associated with Symptom Burden in Patients with Hashimoto's Thyroiditis: A Cross-Sectional Study. *Immunol Invest, 48*, 198-209.

Baxter, M., & Ray, D. W. (2020). Circadian rhythms in innate immunity and stress responses. *Immunology, 161*(4), 261-267.

Boivin, D. B., James, F. O., Wu, A., Cho-Park, P. F., Xiong, H., & Sun, Z. S. (2003). Circadian clock genes oscillate in human peripheral blood mononuclear cells. *Blood, 102*(12), 4143-4145.

Botelho, I. M. B., Moura Neto, A., Silva, C. A., Tambascia, M. A., Alegre, S. M., & Zantut-Wittmann, D. E. (2018). Vitamin D in Hashimoto's thyroiditis and its relationship with thyroid function and inflammatory status. *Endocr J, 65*(10), 1029-1037.

Burnett, R. W., Christiansen, T. F., Covington, A. K., Fogh-Andersen, N., Külpmann, W. R., Lewenstam, A., . . . & Zijlstra, W. G. (2000). IFCC recommended reference

method for the determination of the substance concentration of ionized calcium in undiluted serum, plasma or whole blood. *Clin Chem Lab Med, 38*(12), 1301-1314.

Cajochen, C., Kräuchi, K., & Wirz-Justice, A. (2003). Role of melatonin in the regulation of human circadian rhythms and sleep. *J Neuroendocrinol, 15*(4), 432-437.

Chen, W., & Baler, R. (2000). The rat arylalkylamine N-acetyltransferase E-box: differential use in a master vs. a slave oscillator. *Brain Res Mol Brain Res, 81*(1-2), 43-50.

Chuffa, L. G., Alves, M. S., Martinez, M., Camargo, I. C., Pinheiro, P. F., Domeniconi, R. F., . . . & Martinez, F. E. (2016). Apoptosis is triggered by melatonin in an in vivo model of ovarian carcinoma. *Endocr Relat Cancer, 23*(2), 65-76.

Díez, J. J., Hernanz, A., Medina, S., Bayón, C., & Iglesias, P. (2002). Serum concentrations of tumour necrosis factor-alpha (TNF-alpha) and soluble TNF-alpha receptor p55 in patients with hypothyroidism and hyperthyroidism before and after normalization of thyroid function. *Clin Endocrinol (Oxf), 57*(4), 515-521.

Drugarin, D., Negru, S., Koreck, A., Zosin, I., & Cristea, C. (2000). The pattern of a T(H)1 cytokine in autoimmune thyroiditis. *Immunol Lett, 71*(2), 73-77.

Ertosun, M. G., Kocak, G., & Ozes, O. N. (2019). The regulation of circadian clock by tumor necrosis factor alpha. *Cytokine Growth Factor Rev, 46*, 10-16.

Faria, M., Domingues, R., Paixão, F., Bugalho, M. J., Matos, P., & Silva, A. L. (2020). TNFα-mediated activation of NF-κB downregulates sodium-iodide symporter expression in thyroid cells. *PLoS One, 15*(2), e0228794.

Fröhlich, E., & Wahl, R. (2019). The forgotten effects of thyrotropin-releasing hormone: Metabolic functions and medical applications. *Front Neuroendocrinol, 52*, 29-43.

Giuliani, C., Bucci, I., & Napolitano, G. (2018). The Role of the Transcription Factor Nuclear Factor-kappa B in Thyroid Autoimmunity and Cancer. *Front Endocrinol (Lausanne), 9*, 471.

Gowayed, M. A., Mahmoud, S. A., El-Sayed, Y., Abu-Samra, N., & Kamel, M. A. (2020). Enhanced mitochondrial biogenesis is associated with the ameliorative action of creatine supplementation in rat soleus and cardiac muscles. *Exp Ther Med, 19*(1), 384-392.

Grillone, R., Grimaldi, E., Scopacasa, F., & Dente, B. (2014). Evaluation of the fully automated hematological analyzer Mindray BC 6800: comparison with Horiba ABX Pentra DX120. *Int J Lab Hematol, 36*(4), e55-58.

Gutierrez-Monreal, M. A., Cuevas-Diaz Duran, R., Moreno-Cuevas, J. E., & Scott, S. P. (2014). A role for 1α,25-dihydroxyvitamin d3 in the expression of circadian genes. *J Biol Rhythms, 29*(5), 384-388.

Hong, H. K., Maury, E., Ramsey, K. M., Perelis, M., Marcheva, B., Omura, C., . . . & Bass, J. (2018). Requirement for NF-κB in maintenance of molecular and behavioral circadian rhythms in mice. *Genes Dev, 32*(21-22), 1367-1379.

Ihnatowicz, P., Wątor, P., Gębski, J., Frąckiewicz, J., & Drywień, M. E. (2021). Are Nutritional Patterns among Polish Hashimoto Thyroiditis Patients Differentiated Internally and Related to Ailments and Other Diseases? *Nutrients, 13*, 3675.

Ikegami, K., Refetoff, S., Van Cauter, E., & Yoshimura, T. (2019). Interconnection between circadian clocks and thyroid function. *Nat Rev Endocrinol, 15*, 590–600.

Intan, N. S., Thambiah, S., Hannah, P., NorBaizurah, B., & Baizurah, M. H. (2014). Performance characteristics of anti- thyroid peroxidase and anti- thyroglobulin assays on roche cobas E411 immunoassay system. *Int J Public Health Clin Sci, 1*, 2289-7577.

Kazerouni, F., & Amirrasouli, H. (2012). Performance characteristics of three automated immunoassays for thyroid hormones. *Caspian J Intern Med, 3*(2), 400-104.

Khatiwada, S., Gelal, B., Baral, N., & Lamsal, M. (2016). Association between iron status and thyroid function in Nepalese children. *Thyroid Res, 9*, 2.

Kim, M., Kim, B. H., Lee, H., Jang, M. H., Kim, J. M., Kim, E. H., . . . & Kim, I. J. (2020). Association between Serum Free Thyroxine and Anemia in Euthyroid Adults: A Nationwide Study. *Endocrinol Metab (Seoul), 35*, 106-114.

Kuzmenko, N. V., Tsyrlin, V. A., Pliss, M. G., & Galagudza, M. M. (2021). Seasonal variations in levels of human thyroid-stimulating hormone and thyroid hormones: a meta-analysis. *Chronobiol Int, 38*, 301–317.

Lin, Y., He, L., Cai, Y., Wang, X., Wang, S., & Li, F. (2024). The role of circadian clock in regulating cell functions: implications for diseases. *MedComm (2020), 5*(3), e504.

Liontiris, M. I., & Mazokopakis, E. E. (2017). A concise review of Hashimoto thyroiditis (HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients.Points that need more investigation. *Hell J Nucl Med, 20*(1), 51-56.

Liu, M., Murphy, E., & Amerson, E. H. (2016). Rethinking screening for thyroid autoimmunity in vitiligo. *J Am Acad Dermatol, 75*(6), 1278-1280.

Mani, V., Natarajan, M., & Mohanakrishnan, V. (2019). Comparison of Total and Ionic Calcium in Hypothyroidism. *J Clin Diagnostic Res, 13*, 10-12.

Mardente, S., Zicari, A., Consorti, F., Mari, E., Di Vito, M., Leopizzi, M., . . . & Antonaci, A. (2010). Cross-talk between NO and HMGB1 in lymphocytic thyroiditis and papillary thyroid cancer. *Oncol Rep, 24*(6), 1455-1461.

Masters, A., Pandi-Perumal, S. R., Seixas, A., Girardin, J. L., & McFarlane, S. I. (2014). Melatonin, the Hormone of Darkness: From Sleep Promotion to Ebola Treatment. *Brain Disord Ther, 4*(1).

McLachlan, S. M., & Rapoport, B. (2004). Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies? *Thyroid, 14*(7), 510-520.

Milo, T., Korem Kohanim, Y., Toledano, Y., & Alon, U. (2023). Autoimmune thyroid diseases as a cost of physiological autoimmune surveillance. *Trends Immunol, 44*(5), 365-371.

Milon, A., Pawlicki, P., Rak, A., Mlyczynska, E., Płachno, B. J., Tworzydlo, W., . . . & Kotula-Balak, M. (2019). Telocytes are localized to testis of the bank vole (Myodes glareolus) and are affected by lighting conditions and G-coupled membrane estrogen receptor (GPER) signaling. *Gen Comp Endocrinol, 271*, 39-48.

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., . . . & Sassone-Corsi, P. (2008). The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell, 134*(2), 329-340.

Ohmori, M., Harii, N., Endo, T., & Onaya, T. (1999). Tumor necrosis factor-alpha regulation of thyroid transcription factor-1 and Pax-8 in rat thyroid FRTL-5 cells. *Endocrinology, 140*(10), 4651-4658.

Ongphiphadhanakul, B., Fang, S. L., Tang, K. T., Patwardhan, N. A., & Braverman, L. E. (1994). Tumor necrosis factor-alpha decreases thyrotropin-induced 5'-deiodinase activity in FRTL-5 thyroid cells. *Eur J Endocrinol, 130*(5), 502-507.

Ordoñez, R., Carbajo-Pescador, S., Prieto-Dominguez, N., García-Palomo, A., González-Gallego, J., & Mauriz, J. L. (2014). Inhibition of matrix metalloproteinase-9 and nuclear factor kappa B contribute to melatonin prevention of motility and invasiveness in HepG2 liver cancer cells. *J Pineal Res, 56*(1), 20-30.

Pang, X. P., Hershman, J. M., Chung, M., & Pekary, A. E. (1989). Characterization of tumor necrosis factor-alpha receptors in human and rat thyroid cells and regulation of the receptors by thyrotropin. *Endocrinology, 125*(4), 1783-1788.

Peliciari-Garcia, R. A., Bargi-Souza, P., Young, M. E., & Nunes, M. T. (2018). Repercussions of hypo and hyperthyroidism on the heart circadian clock. *Chronobiol Int, 35*(2), 147-159.

Peng, S., Li, C., Wang, X., Liu, X., Han, C., Jin, T., . . . & Teng, W. (2016). Increased Toll-Like Receptors Activity and TLR Ligands in Patients with Autoimmune Thyroid Diseases. *Front Immunol, 7*, 578.

Pyzik, A., Grywalska, E., Matyjaszek-Matuszek, B., & Roliński, J. (2015). Immune disorders in Hashimoto's thyroiditis: what do we know so far? *J Immunol Res, 2015*, 979167.

Qu, H., Lin, K., Wang, H., Wei, H., Ji, B., Yang, Z., . . . & Deng, H. (2017). 1,25(OH)(2) D(3) improves cardiac dysfunction, hypertrophy, and fibrosis through PARP1/SIRT1/mTOR-related mechanisms in type 1 diabetes. *Mol Nutr Food Res, 61*(5).

Reale, C., Zotti, T., Scudiero, I., Vito, P., & Stilo, R. (2018). The NF-κB Family of Transcription Factors and Its Role in Thyroid Physiology. *Vitam Horm, 106*, 195-210.

Reilly, D. F., Westgate, E. J., & FitzGerald, G. A. (2007). Peripheral circadian clocks in the vasculature. *Arterioscler Thromb Vasc Biol, 27*(8), 1694-1705.

Saida, F. B., Padilla-Chee, M., Dou, C., & Yuan, C. (2018). First two-reagent vitamin D assay for general clinical chemistry. *Clin Biochem, 55*, 28-35.

Salem, T. M., Abdelmonem, E., & Fayad, A. (2021). Hashimoto's thyroiditis, iron, and vitamin D deficiency among Egyptian female patients: associations and possible causalities. *Hormones (Athens), 20*(4), 833-836.

Sarkar, R. (2014). TSH Comparison Between Chemiluminescence (Architect) and Electrochemiluminescence (Cobas) Immunoassays: An Indian Population Perspective. *Indian J Clin Biochem, 29*(2), 189-195.

Shahid, M. A., Ashraf, M. A., & Sharma, S. (2025). Physiology, Thyroid Hormone *StatPearls*. Treasure Island (FL) ineligible companies. Disclosure: Muhammad Ashraf declares no relevant financial relationships with ineligible companies. Disclosure: Sandeep Sharma declares no relevant financial relationships with ineligible companies.: StatPearls Publishing Copyright © 2025, StatPearls Publishing LLC.

Shen, Y., Endale, M., Wang, W., Morris, A. R., Francey, L. J., Harold, R. L., . . . & Liu, A. C. (2021). NF-κB modifies the mammalian circadian clock through interaction with the core clock protein BMAL1. *PLoS Genet, 17*, e1009933.

Susanna, T. Y., Sagayaraj, A., Shashidhar, K. N., Gomathi, M., & Mahesh, V. (2016). A correlative study of thyroid profile and mineral status in patients with hypothyroidism - A hospital-based case control study. *Asian J Pharm Clin Res, 9*, 292-294.

Tan, D. X., Manchester, L. C., Sanchez-Barcelo, E., Mediavilla, M. D., & Reiter, R. J. (2010). Significance of high levels of endogenous melatonin in Mammalian cerebrospinal fluid and in the central nervous system. *Curr Neuropharmacol, 8*(3), 162-167.

Tandon, N., Yan, S. L., Morgan, B. P., & Weetman, A. P. (1994). Expression and function of multiple regulators of complement activation in autoimmune thyroid disease. *Immunology, 81*(4), 643-647.

Tipu, H. N., Ahmed, D., Bashir, M. M., & Asif, N. (2018). Significance of Testing Anti-Thyroid Autoantibodies in Patients with Deranged Thyroid Profile. *J Thyroid Res, 2018*, 9610497.

Vriend, J., & Reiter, R. J. (2015). Melatonin feedback on clock genes: a theory involving the proteasome. *J Pineal Res, 58*(1), 1-11.

Zeng, Y., Guo, Z., Wu, M., Chen, F., & Chen, L. (2024). Circadian rhythm regulates the function of immune cells and participates in the development of tumors. *Cell Death Discov, 10*, 199.

Zhu, Q., Su, J., Wang, X., Tang, M., Gao, Y., & Zhang, D. (2020). Serum concentrations of TNF-α and its soluble receptors in Graves' disease. *Endocr Connect, 9*(7), 736-746.