EVALUATION OF SERUM MELATONIN IN THYROID DISORDERS

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ABSTRACT
In order to evaluate the pineal-thyroid-pituitary interaction, the serum melatonin concentration was estimated in 45 age and sex matched subjects: 20 with hyperthyroidism, 12 with hypothyroidism and 13 with euthyroid function. Serum melatonin as well as serum T3, T4, and TSH were determined by enzyme immunoassay. A significant decrease in serum melatonin was observed in hyperthyroid patients whereas there was a significant increase of serum melatonin in hypothyroid patients. Also, there was a significant negative correlation between serum melatonin and serum T4 in all subjects. On the other hand, there was a significant positive correlation between serum melatonin and serum TSH in euthyroid individuals. In conclusion our study demonstrated that patients with hyperthyroidism secrete less melatonin than euthyroid subjects and this observation may help in ameliorating the anxiety symptoms that accompany thyrotoxicosis.

INTRODUCTION
Shortly after its discovery, the pineal hormone melatonin was functionally linked to the regulation of the neuroendocrine axis, particularly to the reproductive system. Melatonin (N-acetyl-5-methoxy-tryptamine) is a neurohormone synthesized by the pineal gland from amino acid tryptophan through serotonin (5-hydroxytryptamine) as intermediate (Rodwell, 1996). The role exerted by melatonin in human physiology has not been completely ascertained (Cagnacci, 1996). It has been suggested as a potent antioxidant that may protect against development of atherosclerosis and cancer: however, these effects
are unproven and controversial (Abuja et al., 1997; Duell et al., 1998).

On the other hand, Poeggeler et al. (1993) and Reiter (1995) found that melatonin is a very potent and efficient endogenous free radicals scavenger. The antioxidant activities of melatonin have been well documented in tissue homogenates and organisms as well. When rats are treated with the chemical carcinogen safrole, this agent induces the generation of free radicals which in turn extensively damage nuclear DNA; this damage is almost eliminated if the animals are co-treated with melatonin (Reiter, 1995).

Also, damage to DNA in human lymphocytes due to ionizing radiation, which is known to induce free radical formation, is greatly reduced if the cells are treated with melatonin prior to radiation. Cytosolic protein seems also to be protected from free radical damage when melatonin is present. Also, membrane lipid peroxidation, induced either in vivo or in vitro by any of several means, all of which involve free radicals, is drastically attenuated in the presence of melatonin (Greenstock, 1993; Reiter, 1995). Also, Kelly and Loo (1997) concluded that physiological concentrations of melatonin can effectively inhibit oxidative stress.

Lesnikov and Pierpaoli (1994) suggested the presence of an endogenous "aging clock" in the pineal gland. So, the replacement of pineal gland from an old mouse with the pineal gland from a young donor mouse remarkably prolongs its life and conversely, the old pineal gland transplanted into a younger mouse will considerably shorten its life span.

The changing pattern of nocturnal melatonin production was found to be the signal for the annual cycle of reproduction in photoperiodic species. Since then, melatonin also has been linked to circadian rhythms, immune function, sleep, retinal physiology and endocrinal functions in general. In recent years, however, the sphere of melatonin was further expanded (Reiter and Carneiro, 1997).

Pineal-thyroid interaction has been well documented in a number of studies carried out in experimental animals (Vriend, 1983), but not in humans. Melatonin, the main pineal indolamine, has been shown to exhibit an inhibitory effect upon thyroid
function in rats (Singh & Turner, 1972) and Syrian hamsters (De-Fronzo & Roth, 1972; Vaughan et al., 1984; Vriend & Wassermann, 1986). Melatonin exerts this effect probably at the hypothalamic level by lowering TRH release (Vriend & Wilber, 1983), but a direct influence on the pituitary and thyroid function is also possible (Lewinski et al., 1987). On the other hand, the effect of thyroid hormones upon pineal activity has not been extensively studied (Soszynski et al., 1988). Champney et al. (1985) reported that alteration of thyroid homeostasis in experimental animals leads to a decrease in the pineal melatonin content during the night. In humans, as in all mammals, melatonin is secreted with a circadian rhythm. Serum concentrations of this hormone are low in the day and several times higher during the night (Vaughan, 1984).

The circadian rhythm of melatonin secretion seems to be unaltered in patients with hypo or hyper-thyroidism (Soszynsky et al., 1988). On the other hand, studies in animals suggest that melatonin exerts an inhibitory effect on thyroid gland weight (Pazo et al., 1968) and function (Singh et al., 1969).

Relationships between melatonin secretion and pituitary-thyroid function are still discussed (Soszynsky et al., 1988; Reiter, 1991; Bellastella et al., 1995).

The aim of this work is to study the level of pineal body hormone, melatonin, in hyperthyroid, hypothyroid and euthyroid subjects to find any possible correlation with triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH).

SUBJECTS AND METHODS
This study was conducted on 45 subjects (36 women and 9 men). They were classified into three groups according to the thyroid status:

I. Hyperthyroid Group Comprised 20 patients (16 women & 4 men). Their ages ranged from 26 to 40 years.

II. Hypothyroid Group Comprised 12 patients (9 women & 3 men). Their ages ranged from 24 to 40 years.

III. Euthyroid Group Comprised 13 subjects (11 women & 2 men). Their ages ranged from 26 to 38 years.

Apart from thyroid diseases the patients did not show any other endocrine disorders. No medication was
used at least one week prior to the study. All subjects were maintained under standard hospital conditions with sleep hours between 10.00 pm and 06.00 am. The patients were selected from those attending the Endocrine Surgical Unit while the euthyroid group was selected from subjects attending The General Surgery Departments for doing minor surgical non-endocrinal operations. Informed consents were obtained from all the participants. Two blood specimens were taken during a 24 h period, a morning sample at 06.00 am and an evening sample at 10.00 pm. Serum was separated using cooling centrifuge, divided into aliquots and stored frozen at 
-20°C until assay. The morning samples were used for estimation of melatonin, T3, T4 and TSH. Whereas the evening samples were used for determination of melatonin levels.

- Serum melatonin was determined by enzyme linked immunosorbent assay according to the method of Arendt (1985) using the kits from IBL Gesellschaft Fur Immunchemie and Immunobiologie MBH, Flughafenstrabe, Humburg.

- Serum T3, T4 and TSH were determined by enzyme linked immunosorbent assay using the kits from Diagnostic Systems Laboratories Inc., Webster, Texas, USA.

**Statistical Analysis**

The data of this study were recorded and calculated on an IBM compatible personal computer using SPSS/PC computer package version 5 (SPSS Inc. Chicago IL). Comparisons between groups were calculated using the Mann-Whitney-μ test (Daly et al., 1992). Also, Spearman's correlation coefficient (r) was used to study the correlations between morning and evening serum melatonin, T3, T4 and TSH levels. A value of P < 0.05 was considered significant.

**RESULTS**

Table (1) reveals that there is a significant increase of serum T3 (P<0.001) and serum T4 (P < 0.001) in hyperthyroid group compared to euthyroid one. Also there is a significant decrease of serum TSH (P<0.001) in hyperthyroid group compared to euthyroid one. On the other hand, there is a significant decrease of serum T3 (P<0.001) and serum T4 (P<0.001) and significant increase of serum TSH (P<0.001) in hypothyroid group when compared to euthyroid one.

As regards serum melatonin lev-
els, table (1) reveals a significant decrease of morning (P<0.001) and evening (P<0.001) levels in hyperthyroid patients when compared to either hypothyroid or euthyroid subjects. Whereas there is a significant increase of morning melatonin (P<0.01) and evening melatonin (P=0.012) in hypothyroid group compared to euthyroid one.

It is clear from table (2) that serum melatonin levels are negatively correlated with serum T4 levels in all groups. Whereas there is a significant positive correlation between serum melatonin and serum TSH in euthyroid group only.

Table (1): Comparison of the levels of different hormones in the studied groups.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Group I Hyperthyroid</th>
<th>Group II Hypothyroid</th>
<th>Group III Euthyroid</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I vs II</td>
<td>I vs III</td>
<td>II vs III</td>
<td></td>
</tr>
<tr>
<td>T3 ng/ml</td>
<td>2.72±0.21</td>
<td>0.51±0.06</td>
<td>1.13±0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>T4 µg/dl</td>
<td>15.51±0.58</td>
<td>2.74±0.31</td>
<td>7.1±0.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>TSH µIU/ml</td>
<td>0.30±0.08</td>
<td>22.15±3.28</td>
<td>2.09±0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<td></td>
</tr>
<tr>
<td>Morning MT pg/ml</td>
<td>2.45±0.09</td>
<td>19.86±1.88</td>
<td>9.28±1.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Evening MT pg/ml</td>
<td>8.34±1.14</td>
<td>50.05±6.53</td>
<td>29.91±5.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.012</td>
<td></td>
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</tbody>
</table>

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Table (2): Correlation between serum melatonin levels and other hormones in the studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I Hyperthyroid</th>
<th>Group II Hypothyroid</th>
<th>Group III Euthyroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃</td>
<td>-0.050</td>
<td>-0.190</td>
<td>0.146</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>T₄</td>
<td>-0.548</td>
<td>-0.726</td>
<td>-0.434</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH</td>
<td>0.081</td>
<td>-0.095</td>
<td>0.482</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

P > 0.05 = non significant.

**DISCUSSION**

It is well documented that administration of pharmacological doses of melatonin (MT) to both laboratory animals and humans produces hypnotic-like effects (Vollrath et al., 1981; Arendt, 1988), and it has been maintained that MT may participate in the physiological regulation of sleep and sleepiness (Redman et al., 1983; Wurtman & Lieberman, 1985). Sleep disturbances are known to prevail in certain disease states. Hyperthyroidism is for example associated with insomnia (Lidz, 1971; Spaulding & Utiger, 1981), whereas somnolence is common in hypothyroidism (Adams & Rosman, 1971; Spaulding & Utiger, 1981). Whether these sleep disturbances are caused by changes in nocturnal MT secretion, or by other factors, has previously not been given much attention (Rojdmark et al., 1991).

The data of this work revealed a significant increase in the level of serum melatonin in hypothyroid patients as compared to euthyroid individuals whereas there was a significant decrease in serum melatonin level in hyperthyroid patients when compared to euthyroid subjects. These results agree partially with the results of Rojdmark et al., (1991) who reported that patients with hypothyroidism have higher total serum nocturnal melatonin secretion than normal individu-
als. However, thyrotoxic patients did not differ from normal individuals. Meanwhile, the same authors suggested that both hypothyroid and thyrotoxic patients have disturbed pineal function. Although these findings support the hypothesis that thyroid hormones might inhibit the pinealocytes, results from other studies strongly oppose it, where Nir & Hirschmann (1978) postulated that T3 and T4 have a stimulatory influence and Reiter (1982) suggested that thyroidectomy has an inhibitory effect on MT secretion. Furthermore, results obtained by Rojdmark et al. (1991) showing that thyrotoxic patients and normal subjects secrete similar amounts of MT during the night, strongly suggest that factors other than the thyroid hormones cause the increased nocturnal MT secretion in patients with hypothyroidism.

Lewy (1983) suggested that melatonin circadian secretion has been considered a biological marker of the pineal adrenergic system activity. Noradrenaline is one of the possible factors that are responsible for the increased MT secretion in hypothyroidism; since its release from post-ganglionic sympathetic nerves and interaction with β-adrenergic receptors on the pinealocyte membrane, initiates a cascade of intracellular events, which results in synthesis and release of melatonin (Reiter, 1988).

It has been maintained that patients with hypothyroidism have elevated plasma noradrenaline levels (Christensen, 1972), whereas these levels are either normal, or slightly decreased, in patients with thyrotoxicosis (Christensen, 1973), although many of the symptoms of thyrotoxicosis are induced by increased adrenergic stimulation (Green, 1987). However, those changes in adrenergic activity exerted no marked effect on melatonin rhythm in the patients with thyroid disorders and this finding supports the observation that general sympathetic stimulation does not alter the melatonin secretion in humans (Vaughan, 1984; Soszynski et al., 1988).

In fact, neither human pinealocytes (Moore et al., 1979), nor pinealocytes from Syrian hamsters (Lipton et al., 1982), respond significantly to exogenous administration of β-agonists during the day. On the other hand, when β-receptor agonists (noradrenaline and isoproterenol) are given to Syrian hamsters during the
night, their pineal glands respond with increased melatonin production (Reiter et al., 1987). The implication of these findings is that up-regulation of pinealocyte receptors may occur during the night in Syrian hamsters. Whether nocturnal up-regulation of the β-adrenergic receptors also occurs in humans is unknown and this may be what occurs in patients with hypothyroidism (Rojdmark et al., 1991). Such a mechanism seems to be compatible with the observation that hypothyroid patients secrete more melatonin during the night than normal individuals.

In animals, the signal for the circadian MT rhythm originates in the suprachiasmatic nucleus (Moore, 1978) and there is a good reason to believe that this signal has a similar origin in man (Frazer et al., 1986). Normal subjects display MT secretion peaks around 2.00 to 4.00 am (Arendt, 1988; Rojdmark & Wetterberg, 1989), but phase advanced secretion peaks have been found in association with mental depression (Wetterberg et al., 1984). It is possible that thyrotoxic patients and those with mental depression have a factor in common, which influences the suprachiasmatic signal and brings about a shift in the MT secretion peak (Rojdmark et al., 1991).

Nir & Hirschmann (1978) investigated the in vitro effect of thyroid hormones on the rat pineal metabolism. They found that lower concentrations of T3 and T4 increased N-acetyloserotonin and melatonin synthesis, but that stimulation disappeared after the addition of high amounts of thyroid hormones to the pineal cell culture.

In the present study we found that melatonin concentrations were negatively correlated with T4 in all groups. Except for a positive correlation with TSH in the euthyroid group, no significant correlations were found between serum melatonin and T3 and TSH in other groups. These results are in agreement with Rao & Mager (1987) who reported a positive correlation between melatonin and TSH levels in healthy subjects. On the other hand, our results are contradictory with the results of Soszynski et al. (1988) who reported a positive correlation between MT and TSH in hypothyroid patients and a negative correlation between serum melatonin and T3 in hyperthyroid patients. This controversy as regards the correlations between serum melatonin on one side
and T₃, T₄ and TSH on the other side suggests that there is another factor(s) controlling the pituitary-thyroid-pineal axis other than the simple feedback mechanism which needs to be elucidated. In conclusion our study demonstrated that patients with hyperthyroidism secrete less melatonin than euthyroid subjects and this observation could encourage the use of melatonin therapy in cases of thyrotoxicosis aiming to ameliorate the anxiety symptoms that accompany these disorders.

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EVALUATION OF SERUM MELATONIN IN THYROID etc...


تقييم مستوى الميلاتونين في حالات إضطرابات الغدة الدرقية

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لتقييم الارتباط التفاعلي للغدة الدرقية والغدة النخامية تم قياس مستوى الميلاتونين في مصل 45 شخصًا في نفس المرحلة العمرية وتم تقسيمهم إلى ثلاثة مجموعات: الأولى مكونة من 20 شخصًا يعانون من فرط إفراز الغدة الدرقية، الثانية مكونة من 12 شخصًا يعانون من نقص إفراز الغدة الدرقية، الثالثة مكونة من 13 شخصًا ذوي إفراز طبيعي للغدة الدرقية. وقد تم قياس مستوى كل من هرمون الغدة الدرقية (TSH) وأيضًا أبيوبروترودين والهرمون المحفز للغدة الدرقية بالإضافة إلى هرمون الميلاتونين في مصل هؤلاء الأشخاص بطريقة الألبيزا. وقد أسفرت نتائج هذا البحث عن تقصي معنوي في مستوى الميلاتونين في مصل مرضى فرط إفراز الغدة الدرقية بينما وجدت زيادة معنوية في مستوى الميلاتونين في مصل مرضى نقص إفراز الغدة الدرقية. كذلك وجدت علاقة سلبية ذات دلالة إحصائية بين مستوى الميلاتونين والهرمون المحفز للغدة الدرقية في مصل الأشخاص ذوي الأنسجة الطبيعية للغدة الدرقية. ومستهدف من هذا البحث أن إفراز الغدة الدرقية يسبب نقص في مستوى الميلاتونين وهذا قد يشجع على إضافة الميلاتونين كعلاج مساعد في حالات فرط إفراز الغدة الدرقية لتخفيف أعراض الفلق والاضطراب المصاحبة لهذا المرض.