Serum Levels of Melatonin and Cytokines in Multiple Sclerosis

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Cytokines are important factors of the immune system in autoimmune diseases such as multiple sclerosis (MS) in which damage caused by oxidants plays a major role in the pathology. Melatonin secreted by the pineal gland has recently been considered as an antioxidant. The purpose of this study was to determine the relationship between melatonin and cytokines in patients with MS. Thirty patients with MS and 30 healthy controls were selected. Serum levels of melatonin and cytokines, including interleukin-4, interferon-γ, and tumor necrosis factor alpha (TNF-α), were detected in all participants by the enzyme-linked immunosorbent assay (ELISA) method. There was a significant difference between patient and control groups in the levels of melatonin and TNF-α. Also, no significant correlation between the serum levels of melatonin and cytokines in both patient and control groups was seen. We concluded that decrease of melatonin and subsequent increase of pro-inflammatory cytokine, TNF-α, could be a factor in the inflammatory reactions in the pathologic process of MS. (*Biomed J 2014;37:90-92*)

Key words: cytokines, immune system, melatonin, multiple sclerosis, pineal gland

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS), in which the myelin sheath around nerve fibers is destroyed in multiple parts of the CNS, causing various disorders including dysfunction of the optic nerve, the brain stem, the spinal cord, and the cerebellum.[1] Immune and inflammatory factors have been known effective in this disease, and damage due to oxidants plays a key role in its pathology.[2] Cytokines are important immune factors in autoimmune diseases such as MS. Cytokines are proteins that play an important role in the regulation of the body's inflammatory response to foreign agents. These proteins include interleukins [e.g., interleukin-4 (IL-4)], interferons [e.g., interferon gamma (IFN-γ)], and tumor necrosis factors [e.g., tumor necrosis factor alpha (TNF-α)]. IFN-γ has a role in the inflammatory reactions and its increase can lead to autoimmune diseases. IL-4 is another cytokine that fights against inflammation. Another cytokine that participates in the local inflammation process is TNF-α.[3] Melatonin hormone is secreted by the pineal gland. It has recently been proposed as an antioxidant and its effects on the immune system have been studied.[4] There are reports of abnormal daily melatonin profiles in a number of psychiatric and neurological disorders, but the significance of such abnormalities is far from clear.[5] Melatonin has a major importance in protecting the brain from oxidative/nitrosative neural damages, and has been shown to have efficacy in abating them.[6]

Despite this, information on melatonin, especially its relationship with inflammatory and immune factors mentioned above, is negligible. Moreover, cases about MS remain unidentified and obscure. MS is an autoimmune disease and the results of the present study could possibly be effective in finding solutions to combat it.

METHODS

Thirty patients with MS (24 females and 6 males; mean age 28.20 ± 7.35 years) participated in the present study. Newly diagnosed patients prior to the start of any treatment were enrolled in this study. MS disease was confirmed through magnetic resonance imaging (MRI) by a neurologist. Patients with primary and secondary progressive diseases and also those with acute attack who were on treatment were excluded from the study. Thirty healthy people without inflammatory or autoimmune disease were simply analyzed as normal control subjects (22 females and 8 males; mean age 28.64 ± 8.43 years). Seven milliliters of blood samples was collected from brachial vein at 8:00 AM and serum levels of melatonin (using kits produced by IBL, Hamburg, Germany) and cytokines

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including IL-4, IFN-γ, and TNF-α (using kits produced by Bender Med System, Vienna, Austria) were measured in all of subjects by enzyme-linked immunosorbent assay (ELISA) method. Collected data were analyzed by the SPSS software. Other clinical variables including complete blood count (CBC), triglyceride (TG), and cholesterol were also measured. Statistical significance was calculated using the independent Student's *t* test for comparison between patient and control groups. Pearson correlation test was used for evaluation of relationships. The level of significance in all patients was set at a two-tailed $p < 0.05$.

RESULTS

All data about CBC, TG, and cholesterol were in normal range in both patient and control groups. Results showed that there was a significant difference in the serum levels of melatonin between patient and control groups. The mean values for serum melatonin concentration were 21.33 ± 17.68 and 60.31 ± 51.44 pg/ml in patient and control groups, respectively ($p = 0.001$). Results also showed significant difference in the serum concentrations of TNF-α between patient and control groups. While the mean value for serum levels of TNF-α was 4.13 ± 2.26 in the patient group, it was 1.95 ± 1.27 pg/ml in controls ($p = 0.04$). There was not any significant difference in the serum levels of IL-4 and IFN-γ between patient and control groups [Table 1]. Also, no significant correlation between the serum levels of melatonin and cytokines in both patient and control groups was seen.

DISCUSSION

Researchers have emphasized the role of melatonin as an antioxidant and free radical scavenger in diseases of the nervous system. In addition, melatonin stimulates certain enzymes and, therefore, strengthens the activity of other antioxidant agents. It also exerts anti-inflammatory effects by reducing pro-inflammatory cytokines in diseases of the CNS,[6] while some researchers disagree with this opinion and believe melatonin can increase in some conditions including autoimmune diseases such as rheumatoid arthritis and also can play a role in the immune system as an inflammatory factor.[7]

Table 1: Serum levels of melatonin and cytokines (pg/ml) in patient (MS) and control (healthy) groups (Mean±SD)

Groups	Patient	Control	*p* value
Variables			
Melatonin	21.33±17.68	60.31±51.44	0.001
IL-4	67.77±64.43	70.93±59.64	>0.05
IFN-γ	10.02±8.26	8.54±7.93	>0.05
TNF-α	4.13±2.26	1.95±1.27	0.04

Abbreviations: MS: Multiple sclerosis; TNF: Tumor necrosis factor

In the present study, serum level of melatonin was lower in MS patients compared to healthy controls. This finding is in accordance with the results of Maestroni.[7] However, Haas *et al*., emphasize the high level of melatonin in this disease and suggest that melatonin is an initiating factor in disease progression and attack.[8]

Several factors impair the production of melatonin, including seasonal affective disorder.[9] On the other hand, findings of a study suggest that patients with affective disorder are at higher risk for MS-associated cognitive dysfunction and neuropsychiatric symptoms, a conclusion that has implications for the emerging role of personality in clinical neuroscience.[10] Beta-blockers have been also shown to reduce the production of melatonin via specific inhibition of beta-1 adrenergic receptors. A closer look at the available evidence has cast serious doubts on the safety of beta-blockers for patients with MS. Beta-blockers can lead to an exacerbation of MS symptoms. Various CNS side effects associated with the use of beta-blockers are linked with reduction of melatonin levels.[11]

We also found higher levels of TNF-α in serum of patients with MS than in healthy controls. This finding correlates with results of Robinson *et al*.,[12] and also Selmaj.[13] TNF-α is a pro-inflammatory cytokine that is found over most of the lesions of MS.[14]

In the present study, melatonin was reduced in the autoimmune disease (MS), but no relationship between melatonin and cytokines was observed. According to the results of a case–control study, salivary melatonin level was significantly low among patients with MS after controlling the effect of age. However, the level of melatonin was not affected by the duration of disease, treatment, and sex.[15] Studies in the past two decades have shown that melatonin has an effect on the immune system, which plays its role mostly through the regulation of cytokine production. Thus, melatonin up-regulates the production of IL-2, IFN-γ, and TNF-α.[16,17] *In vivo* studies suggest that melatonin may stimulate the immune system. Other researchers have reported direct effects of melatonin on lymphocytes and indirect effects on cytokines.[17] However, *in vitro* studies have shown conflicting results indicating no effect of melatonin on lymphocytes.[18] The results of a study show that melatonin is able to activate human Th1 lymphocytes by increasing the production of IL-2 and IFN-γ *in vitro*. Th2 cells appear not to be affected by melatonin, since IL-4, which is mostly produced by Th2 cells, is not modified by the hormone.[18] Treatment of lymphocytes with melatonin did not significantly alter lymphocyte proliferation or IL-2 or IFN-γ expression in lymphocytes.[19] The reasons for the apparent contradictions are not clear, but it seems that in low serum levels melatonin

is not able to regulate and affect cytokines. Cytokines play an important role in the pathogenesis of inflammatory diseases including MS. Experimental models have played a critical role in unraveling the roles of individual cytokines in this disease; however, these studies occasionally yield conflicting results, highlighting the complex role that cytokines play in the disease process.[20]

Conclusions

Given these research findings, it can be concluded that melatonin is reduced in MS, which could be an important factor in the pathophysiological changes in this disease. The researchers also concluded that the probable cause of inflammation in MS is increased pro-inflammatory cytokine, TNF-α. However, changes of serum levels of melatonin and TNF-α could not be considered as the primary etiopathogenic factor for the occurrence of MS.

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