Serum Nocturnal Melatonin in Neoplastic and Non-Neoplastic (Non Erosive) Upper Gastrointestinal Disorders

Ehab F Moustafa[1], Sahar M Hassany[1], Essam Abdel Mohsen[2], Amal M Abdel-Aal[3] and Amal A Mahmoud[3]

Departments of Tropical Medicine and Gastroenterology[1], Internal Medicine[2] and Clinical Pathology[3], Faculty of Medicine, Assiut University, Egypt
ehabmostafa_99@yahoo.com

Abstract: Background: Melatonin is a powerful antioxidant and scavenger of free radicals. These properties contribute to its strong anti-inflammatory effect. In addition it was known to be a natural oncostatic compound. **Aim of the work:** to investigate serum nocturnal melatonin in neoplastic and non-neoplastic (non erosive) upper gastrointestinal tract disorders. **Patients and methods:** Fifty-three subjects were included in the study (30 males and 23 females). Sixteen with Cancer stomach, 9 with cancer esophagus, 12 with functional dyspepsia, 6 with NERD and 10 healthy subjects as a control. Serum melatonin was measured at 11 p.m (nocturnal) and 6 a.m. **Results:** Cancer stomach and cancer esophagus patients had significantly lower nocturnal melatonin concentration than control, 29.35 ± 23.85 & 16.01 ± 10.70 versus 97.72 ± 9.41pg/ml ($p<0.5$), while functional Dyspepsia and NERD patients had significantly higher nocturnal melatonin concentration than control, 167.67 ± 88.21 & 197.84 ± 67.02 versus 97.72 ± 9.41pg/ml ($p< 0.5$). Serum nocturnal melatonin in neoplastic upper GIT disorders (cancer stomach and cancer esophagus patients) was significantly lower than in non neoplastic non erosive upper GIT disorders (functional dyspepsia and NERD), 24.55 ± 20.89 versus 177.73 ± 81.05 pg/ml ($p< 0.5$). **Conclusion:** Serum nocturnal melatonin in neoplastic upper GIT disorders (cancer stomach and cancer esophagus patients) was significantly lower than in non neoplastic non erosive upper GIT disorders (functional dyspepsia and NERD), this reduction of nocturnal melatonin supports its role as a natural oncostatic agent. Studying the benefit of adding melatonin to standard treatment of neoplastic upper GIT disorders (surgery, chemotherapy, or radiotherapy) is recommended.
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Key words: nocturnal melatonin, Cancer stomach, cancer esophagus, NERD, Functional dyspepsia.

1. Introduction

Melatonin is a powerful antioxidant and scavenger of free radicals. These properties contribute to its strong anti-inflammatory effect (1). Melatonin administration increased the number and size of Payer's patches, the main component of the GIT immune system (2). Because of these actions, melatonin has been tested as a clinical remedy for prevention or treatment of diseases of the digestive tract, such as pathologies of oral cavity (3), ulceration of esophagus (4,5), duodenum (6), stomach, pancreas and colon (7- 9).

Melatonin (MT), an indole formed enzymatically from L-trytophan (Trp), was first discovered in the bovine pineal gland in 1958 by Lerner *et al.* Melatonin is secreted in response to environmental light/dark cycles which are produced by supra-chiasmatic nuclei (SCN), the major circadian oscillators (10).

Melatonin is the most versatile and ubiquitous hormonal molecule produced not only in the pineal gland but like the pinealocytes, the enteroendocrine (EE) cells in GIT mucosa cells, are highly effective in production of serotonin and are also major source of MT. Melatonin synthesized by these cells which is not stored, but immediately released upon the biosynthesis into the extracellular fluid and circulation, where from it easily crosses cell membranes of various tissues and is excreted into saliva, bile, cerebrospinal fluid, milk and urine (11). These observations were further supported by detection in the gut mucosa enzymes engaged in melatonin synthesis, e.g. hydroxyindole-O-methyltransferase (12) and N-acethyltransferase (13). This is also supported by the observation that after pinealectomy melatonin can be detected in the blood of experimental animals (14).The circadian rhythm with a low light-time level and marked surge at darkness persists in most vertebrates irrespective of whether their organisms are active during the day-time or during the night (15, 16).

In human gastroesophageal reflux disease (GERD) lead to dangerous complications such as chronic esophagitis, esophageal ulcer, stricture, Barrett`s, esophagus or Barrett`s carcinoma. Interestingly, Pereira (5) reported that dietary supplementation containing melatonin and L-tryptophan, which is a substrate for MT biosynthesis in patients with GERD, resulted in complete

remission of GERD symptoms in majority of treated patients.

It is of interest that patients with upper digestive tract disorders such GERD or duodenal ulcer show reduced plasma levels of MT, which suggests that the deficiency of this indole exerts detrimental effects on the upper GIT mucosa (17,18), and complete recovery of GERD symptoms was achieved after treatment with melatonin as dietary supplementation (5).

Melatonin is a powerful direct free radical scavenger as well as an indirect antioxidant (19,20). It protects gastric mucosa against destructive activity of free radicals during the ischaemic reperfussion injury process (21) as well as against stress-induced ulcers (22) and ulcers due to non-steroidal anti-inflammatory drugs and other gastrotoxic agents (23-25).

The aim of our study was to investigate serum nocturnal melatonin in neoplastic and non-neoplastic (non erosive) upper gastrointestinal tract disorders.

2. Material and Methods:

The investigations were carried out in 53 subjects, including 30 men and 23 women, aged 20 -72 years (mean age 52.62 yrs). Diagnosis of the disorders of the upper gastrointestinal tract was based on history taking, endoscopy (and biopsy if needed), esophageal pH-metry was conducted in 6 patients. According to the results of the above diagnostic results five study groups were distinguished:

Group 1: Sixteen Cancer stomach patients, 14 patients were adenocarcinoma and 2 were gastric lymphoma

Group 2: Nine Cancer esophagus patients, all were squamus cell carcinoma

Group 3: Twelve patients with functional dyspepsia. They suffered from chronic or recurrent epigastric pain as a predominant symptom. No macroscopic changes of gastric mucosa were observed in these subjects in endoscopic evaluation. The patients were enrolled to the study group according to Rome III Criteria (Epigastric Pain Syndrome - EPS).

Group 4: Six NERD patients had no endoscopic changes in esophageal mucosa, high resolution esophageal motility was done for them for detection of the site of LES (lower esophageal sphincter) through nasal introduction of solid state catheter with 32 transducer (pressure sensor) spaced 0.8 cm to the esophagus and stomach. The system is plotting graphs with high resolution color topography and pressure wave plotting as well. This software was produced by Medical Measurement System (MMS) Enschede, The Netherlands.

A 24 hours pH monitoring was done using Orion Ambulatory pH recorder. A catheter was introduced through one nostril of the patient into the esophagus. A pH sensor on the catheter was positioned just above the LES (lower esophageal Sphincter) by 5 cm according to the previous motility study. The patient was admitted to hospital and during a 24 hours examination every acid reflux was recorded.

Their results of the 24-hours pH-metry ranged from 19.9 to 96.1 points of DeMeester's score (mean value 38.6 points).

Group 5: Ten clinically healthy persons as a control group

We excluded from the study subjects with other organic diseases, peptic ulcer diseases, neuropsychiatric disorders, past history of surgical treatment, and using non-steroidal anti-inflammatory drugs.

Blood samples were taken from all the studied subjects at 11.00 p.m. and 6.00 a.m. After collection, the blood was centrifuged and serum was stored frozen at -80°C until analysis. Serum Melatonin concentration was measured with competitive ELISA method (Immuno-Biological Laboratories, catalogue RE 54021).

Statistical analysis:

Data are expressed as mean±SD or as number, (%). All the statistical analyses were performed using a statistical software package (SPSS, Version 10.0, Inc., Chicago, IL). t test, ANOVA test, Chi-square test, were used when appropriate. $P<0.05$ based on a two-tailed test was considered statistically significant.

Ethical approval:

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. An ethical approval has already been obtained from our local Ethics Committee (Medical Ethics Committee, Faculty of Medicine, Assiut University). Patients were enrolled after written informed consent was obtained.

3. Results:

Age, sex and serum melatonin at 6 a.m. (M1) and at 11 p.m. (M2) of patients and control are demonstrated in table 1. Mean age of cancer stomach patients (16 patients) was 61.38±7 years, range from 40 to 70 years. Twelve patients were males and 4 were females. Mean age of cancer Esophagus patients (9 patients) was 63.22±6.28 years, range from 53 to 72 years. Seven patients were males and 2 were females. Mean age of functional Dyspepsia patients (12 patients) was 48.67±10.52 years, range from 30 to 60 years. Four patients were males and 8 were females. Mean age of NERD patients (6 patients) was 40.67±14.85 years, range from 20 to 55 years. Two patients were males and 4 were females. Mean age of

healthy control subjects (10) was 41.00±6.99 years, range from 35 to 55 years. Five were males and 5 were females.

Endoscopic and histopatholgic findings are demonstrated in table 2, 16 patients were cancer stomach, 14 were adenocarcinoma and 2 were gastric lymphoma. Nine patients were cancer esophagus, all were squamus cell carcinoma. And 16 patients had normal endoscopy.

Melatonin concentration at 6 a.m. (M1) was significantly higher than that at 11 p.m. (M2) in control healthy group ($p = 0.001$). This also found in cancer stomach group ($p = 0.012$) and cancer esophagus group ($p = 0.034$). There was no significant difference between M1 and M2 in functional dyspepsia group and NERD group (Table 3).

Mean concentration of nocturnal melatonin in healthy subjects was 97.72± 9.41 pg/ml.

Figure 1, shows that in patients with cancer stomach nocturnal melatonin concentration (29.35±23.85 pg/ml) was lower than in controls ($p < 0.05$).

Figure 2, shows that in patients with cancer esophagus nocturnal melatonin concentration (16.01±10.70 pg/ml) was lower than in controls ($p < 0.05$).

Figure 3, shows that in patients with functional dyspepsia nocturnal melatonin concentration (167.67±88.21 pg/ml) was higher than in controls ($p < 0.05$).

Figure 4, shows that in patients with NERD nocturnal melatonin concentration (197.84±67.02 pg/ml) was higher than in controls ($p < 0.05$).

Serum nocturnal melatonin in neoplastic upper GIT disorders (cancer stomach and cancer esophagus patients) was significantly lower than in non neoplastic non erosive upper GIT disorders (functional dyspepsia and NERD), 24.55±20.89 pg/ml versus 177.73±81.05 pg/ml ($p < 0.05$), figure 5.

Figure 6, shows that there was highly significant difference between all groups in serum nocturnal melatonin in cancer stomach and cancer esophagus patients (neoplastic upper GIT disorders) versus functional dyspepsia and NERD (non neoplastic non erosive upper GIT disorders) patients versus healthy control, 24.55±20.89 pg/ml versus 177.73±81.05 pg/ml versus 97.72± 9.41 pg/ml ($p < 0.05$).

Table 1: Age, sex and serum melatonin at 6 a.m. (M1) and at 11 p.m. (M2) of all groups.

		Group 1 (n.16)	Group 2 (n.9)	Group 3 (n.12)	Group 4 (n.6)	Group 5 (n.10)	Total (n.53)
Age	Minimum	40	53	30	20	35	20
	Maximum	70	72	60	55	55	72
	Mean	61.38	63.22	48.67	40.67	41.00	52.62
	±SD	7.00	6.28	10.25	14.85	6.99	12.70
Male	NO	12	7	4	2	5	30
	%	75	77.78	33.33	33.33	50	56.60
Female	NO	4	2	8	4	5	23
	%	25.00	22.22	66.67	66.67	50.00	43.40
m1	Minimum	4.3	4.1	7.14	21.9	87.9	4.1
	Maximum	196.7	82.2	239.6	290	200	290
	Mean	73.13	39.00	108.25	149.10	168.79	101.94
	±SD	64.52	33.83	78.35	99.59	30.69	76.54
m2	Minimum	2.8	4.3	45.3	87.9	85.02	2.8
	Maximum	72.6	32	294	267.4	115	294
	Mean	29.35	16.01	167.67	197.84	97.72	90.38
	±SD	23.85	10.70	88.21	67.02	9.41	84.26

Group 1: Cancer stomach patients, 14 patients were adenocarcinoma and 2 were gastric lymphoma
Group 2: Cancer esophagus patients, all were squamus cell carcinoma
Group 3: Functional dyspepsia   Group 4 NERD (diagnosed by pH metry and myometric study)
Group 5: Control healthy subject   M1: serum melatonin at 6 a.m   M2: serum melatonin at 11 p.m (nocturnal)

Table 2: Endoscopic and histopathologic findings of the patients:

Endoscopic Diagnosis	Number and histopathology
Cancer Stomach	N=16 14 Adenocarcinoma 2 Gastric lymphoma
Cancer Esophagus	N=9 All were squamus cell carcinoma
Normal findings	N=18

Table 3: Serum melatonin at 6 a.m (M1) and at 11 p.m (M2) in different groups

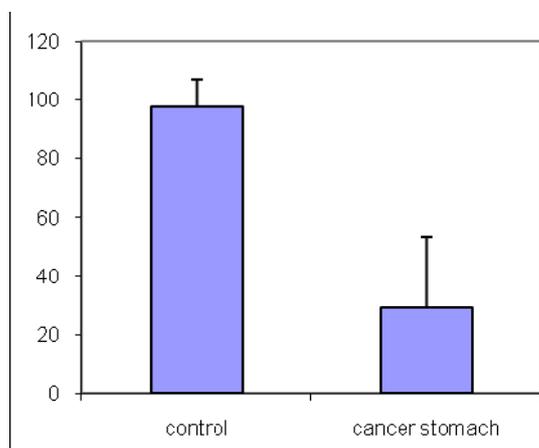|	M1(Mean±SD)	M2(Mean±SD)	Test value	*P*.value
Total	101.94±76.54	90.37±84.25	t=0.915	0.364
Group 1	73.13V±64.53	29.35±23.85	t=2.84	0.012
Group 2	39±33.83	16.01±10.7	t=2.56	0.034
Group 3	108.25±78.35	167.67±88.21	t=-1.76	0.106
Group 4	149.1±99.6	197.84±67.02	t=-0.91	0.403
Group 5	168.79±30.69	97.72±9.41	t=9.02	0.001

Fig. 1: Nocturnal melatonin (11 p.m) in cancer stomach patients versus healthy control (*)
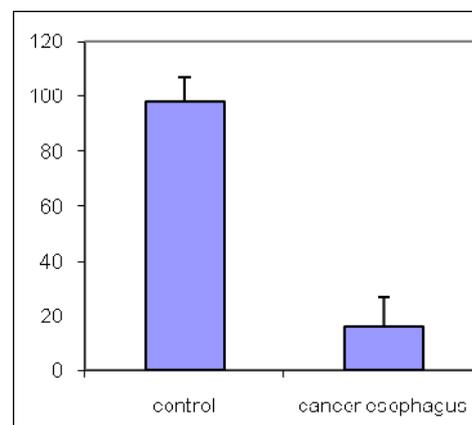

Fig. 2: Nocturnal melatonin (11 p.m) in cancer esophagus patients versus healthy control (*)
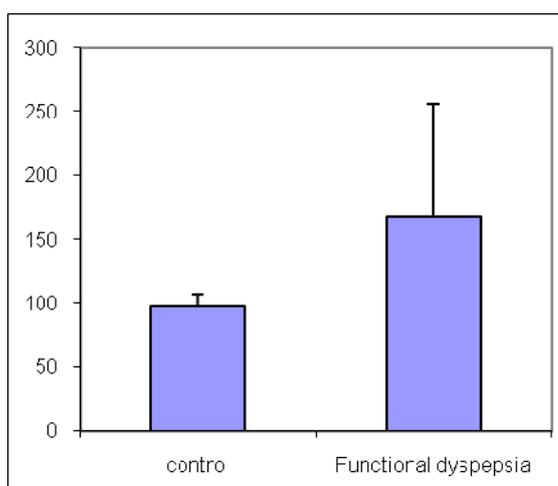

Fig. 3: Nocturnal melatonin (11 p.m) in functional dyspepsia patients versus healthy control (*)
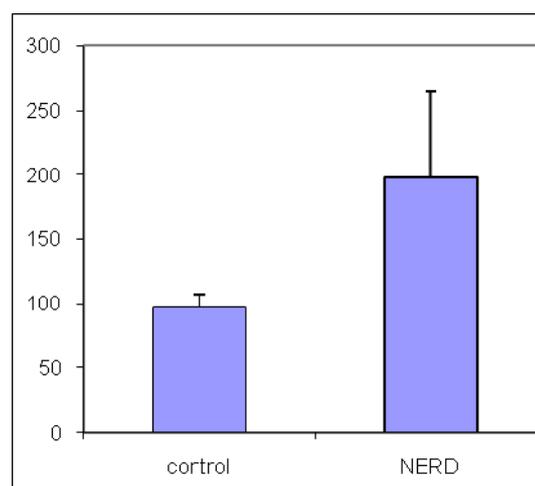

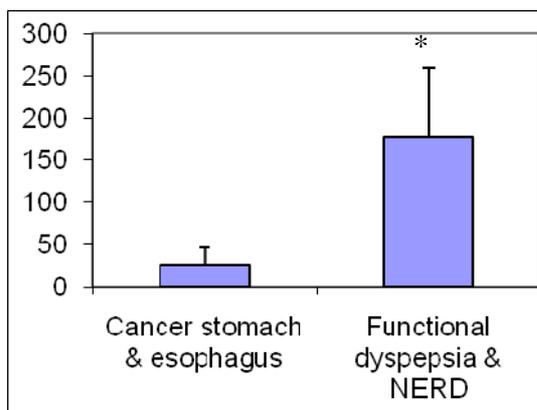**Fig. 4: Nocturnal melatonin (11 p.m) in NERD patients versus healthy control (*)**

Fig. 5: * Highly significant difference in serum nocturnal melatonin (11 p.m) among cancer stomach and cancer esophagus patients (neoplastic upper GIT disorders) versus functional dyspepsia and NERD(non neoplastic non erosive upper GIT disorders) patients
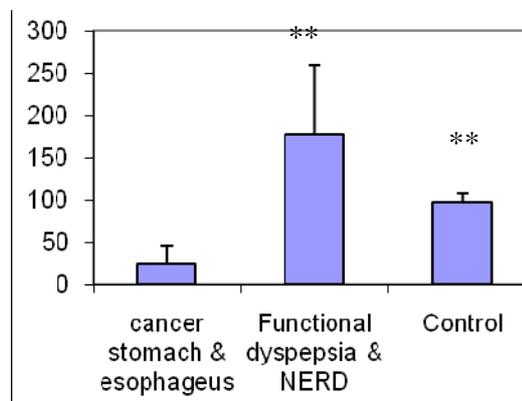

Fig. 6: ** Highly significant difference between all groups in serum nocturnal melatonin (11 p.m) in cancer stomach and cancer esophagus patients (neoplastic upper GIT disorders) versus functional dyspepsia and NERD(non neoplastic non erosive upper GIT disorders) patients versus healthy control

4. Discussion:

Interests of researchers in gastroenterology field are mainly focused on studies on melatonin nocturnal secretion. At these hours the influence of food intake on enterohormones secretion is negligible. On the other hand at bed time the patients often complain of recurrent symptoms of gastrointestinal disorders that to a large extent disturb their sleep and night rest. In some of them phenomenon of nocturnal acid breakthrough (NAB) occurs, still remaining not fully explained (34).

In our study melatonin concentration in blood was measured at two points of time: at 11.00 p.m. (bed time), and 6.00 a.m. Melatonin concentration at 6 a.m. (M1) was significantly higher than that at 11 p.m. (M2) in control healthy group ($p = 0.001$). This also found in cancer stomach group ($p= 0.012$) and cancer esophagus group ($p=0.034$). There was no significant difference between M1 and M2 in functional dyspepsia group and NERD group.

In our study nocturnal serum melatonin in NERD and functional dyspepsia (FD) patients was higher than in control healthy groups.

These results are in agreement with Klupinska *et al.* (34). They suggested that in subjects with NERD and functional dyspepsia (FD) secretion of this hormone remains relatively high. It is not clear why in these patients melatonin secretion is not diminished. May be relatively correct secretion of melatonin is sufficient as an anti-ulcer agent but is insufficient to prevent pyrosis and fasting and nocturnal pain. This further suggests a complex and still unclear mechanism of abdominal symptoms in such patients. Melatonin seems to play a crucial role

in this process. Melatonin has an important property in stimulation of bicarbonate secretion in duodenum. This is particularly important in duodenum where melatonin acts on MT2 receptors of enterocytes (35). It is also suggested that melatonin stimulates duodeno-pancreatic axis and increases secretion of alkaline pancreatic juice (36). The nocturnal reduction in pH in the lumen of stomach and duodenum induces pain and also stimulates EC cells to release an extra portion of melatonin and secondarily bicarbonate secretion by the salivary glands, pancreas and mucosa of the upper digestive tract. This compensatory mechanism is sufficient only in healthy subjects, less protective in NERD and FD, and ineffective in patients with GERD and DU. (34)

Our results showed that in patients with cancer stomach and cancer esophagus nocturnal melatonin concentration was lower than in controls. Also, Serum nocturnal melatonin in neoplastic upper GIT disorders (cancer stomach and cancer esophagus patients) was significantly lower than in non neoplastic non erosive upper GIT disorders (functional dyspepsia and NERD).

In experimental rat models of chemical carcinogenesis the physiological melatonin signal suppresses the initiation phase of tumor genesis. One mechanism by which this may be accomplished is via melatonin's ability to suppress the accumulation of

DNA adducts (the resulting complex when chemicals bind to DNA) formed by carcinogens that cause damage to and permanent alterations in DNA (i.e., mutations and amplifications), which lead to neoplastic transformation. This may be accomplished directly via melatonin's ability to act as a potent free

radical scavenger and/or through its indirect actions to detoxify carcinogens via activation of the glutathione and related antioxidative pathways. In addition to protecting cells from DNA damage, melatonin might also promote the repair of DNA once damage has occurred (37-39).

In experimental models of neoplasia, melatonin, at nocturnal circulating, inhibits the proliferation of human cancer cell lines *in vitro*. This is achieved by delaying the progression of cells through a specific phase of the cell cycle. In some neoplastic cells, this indoleamine acts as a differentiating agent and diminishes their invasive/metastatic potential via alterations in adhesion molecule expression and the support of mechanisms responsible for gap junctional intercellular communication. Additional evidence supports a variety of other biochemical and molecular mechanisms of melatonin's oncostatic action at nocturnal circulating concentrations including the regulation of estrogen receptor (ERa) expression and transactivation, calcium/calmodulin activity, protein kinase C activity, cytoskeletal architecture and function, intracellular redox status, melatonin receptormediated signal transduction cascades, aromatase and telomerase activities, and fatty acid transport and metabolism (37-39).

A major component of physiological melatonin's oncostatic action involves the regulation of the tumor uptake and metabolism of linoleic acid (LA),a an essential omega-6 polyunsaturated fatty acid. Its oncogenic effects, particularly on human breast cancer cells, are related to its ability to upregulate the expression of genes involved in estrogen receptor ERa expression, cell cycle progression, G-protein signaling, and the mitogen-activated protein kinase (MAPK) growth cascade. Like rat hepatomas, human breast cancer xenografts exhibit a day–night rhythm of tumor proliferative activity, LA uptake, and metabolism and signal transduction activity that is driven by the nocturnal, circadian melatonin signal (37,39, 40).

A reduction in endogenous melatonin production leads to immune suppression that may have a stimulatory impact on the development and growth of cancer cells (41,42). This may occur via a reduction in lymphocytes such as natural killer (NK) cells and cytotoxic tumor infiltrating lymphocytes (54,55) as well as a decrease in the production, by circulating immune cells, of a number of cancer-inhibiting cytokines such as interleukin (IL)-2, IL-12, interferon-g (INF-g) (type 1 proinflammatory cytokines produced by Th-1 cells), and tumor necrosis factor (TNF)a (43). Also at physiological nocturnal circulating concentrations, melatonin can reduce the production of IL-10, a type-2 antiinflammatory cytokine that has cancer growth

promoting-activity through its immune suppressive action (44). On the other hand, melatonin can activate human monocytes to produce IL- 6, a cytokine with cancer-stimulatory activity (45). As reviewed above, the direct oncostatic effects of the nocturnal, circadian melatonin signal on a variety of malignancies are well-established; however, the potential mechanisms by which melatonin might indirectly influence the development and growth of cancer via its immunomodulatory actions are much less well understood.

Another route by which endogenous melatonin levels could influence the process of oncogenesis is via the synthesis and release of melatonin from human immunocompetent cells. These cells are not only equipped with the enzymatic machinery to produce substantial concentrations of melatonin but they also express membrane and nuclear melatonin receptors/binding sites that provide the substrates by which endogenous melatonin interacts with the immune system in an endocrine, intracrine, autocrine and/ or paracrine manner to physiologically regulate the IL-2 and IL-2 receptor system in these cells (41, 42). Thus, in addition to melatonin of pineal gland origin, the local release of melatonin from tumor-infiltrating lymphocytes could potentially provide another direct source of oncostatic melatonin to cancer cells. Additionally, lymphocyte derived melatonin might inhibit cancer cell proliferation by stimulating IL-2 through intracrine and/or paracrine mechanisms.

Low levels of melatonin have been associated with breast cancer occurrence and development. Women who work predominantly at night and are exposed to light, which inhibits melatonin production and alters the circadian rhythm, have an increased risk of breast cancer development (46). In contrast, higher melatonin levels have been found in blind and visually impaired people, along with correspondingly lower incidences of cancer compared to those with normal vision, thus suggesting a role for melatonin in the reduction of cancer incidence (47).

Light at night, regardless of duration or intensity, inhibits melatonin secretion and phase-shifts the circadian clock, possibly altering the cell growth rate that is regulated by the circadian rhythm (48). Disruption of circadian rhythm is commonly observed among cancer patients (49,50), and contributes to cancer development and tumor progression (51). Cancer alters neuroendocrine system function in such a way that melatonin levels are lower in patients with non-small-cell lung cancer (52). Indeed, the circadian rhythm of melatonin is also altered in advanced gastrointestinal malignancies, such as colorectal, gastric, and

pancreatic cancer, with respect to healthy humans (53).

The circadian rhythm alone is a statistically significant predictor of survival time for breast cancer patients (54). Several studies have shown that the circadian clock is involved in tumor suppression at the systemic, cellular, and molecular levels, and that cancer should no longer be treated as a local disorder. For instance, the circadian clock regulates the immune response. Disruption of circadian rhythms could therefore lead to immunosuppression, which could disrupt cancer cell immunosurveillance and promote tumor development; however, melatonin as a circadian mediator can target the endogenous clock and has been shown to inhibit immunosuppression (55).

Melatonin can kill directly many different types of human tumor cells (56,57). It is a naturally produced cytotoxin, which can induce tumor cell death (apoptosis) (58). In instances where the tumor has already established itself in the body, melatonin has been shown to inhibit the tumor's growth rate (59,60). Melatonin exhibits natural oncostatic activity and inhibits cancer cell growth (61). In patients in whom cancer already has become a noticeable physical burden and produces overt symptoms, melatonin has been shown to alleviate numerous cancer symptoms (62), and to inhibit development of new tumor blood vessels (tumor angiogenesis), (63) which in turn inhibits the cancer from spreading further (metastasis) (64). Melatonin can retard tumor metabolism and development by lowering the body temperature; it is a natural inducer of hypothermia (65). Furthermore, as an inducer of antioxidants and itself a weak preventive antioxidant, melatonin hinders tumor cells from participating in free radical damage to normal cells and consequently limits oxidative damage to DNA, lipids, amino acids, and proteins (66-68).

Melatonin is so intricately involved in cell regulatory processes that scientists are now studying it as an adjunctive cancer treatment (69). Cancer patients with endogenously depressed melatonin levels may benefit from both meditation and substitutional melatonin therapy, to improve quality of life while potentially inhibiting tumor growth and spread (70).

Conclusion:

Serum nocturnal melatonin in neoplastic upper GIT disorders (cancer stomach and cancer esophagus patients) was significantly lower than in non neoplastic non erosive upper GIT disorders (functional dyspepsia and NERD). Studying the benefit of adding melatonin to standard treatment of

neoplastic upper GIT disorders (chemotherapy and/or radiotherapy) is recommended.

Corresponding authors

Ehab F Moustafa
Departments of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University
Email :ehabmostafa_99@yahoo.com

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