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## NOCTURNAL SECRETION OF MELATONIN IN PATIENTS WITH UPPER DIGESTIVE TRACT DISORDERS

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Recently, the results of many experimental investigations have shown that melatonin possesses gastroprotective properties. On the other hand its role in pathogenesis of upper digestive tract diseases in man still remains unclear. The aim of the study was to investigate nocturnal secretion of melatonin in patients with functional and organic diseases of the upper part of digestive tract. The investigations were carried out in 149 persons, aged 21-51 years, including healthy subjects (group I, n=30), and patients with non-erosive gastroduodenal reflux (NERD, group II, n=24), with gastroesophageal reflux disease (GERD, group III, n=25), with functional dyspepsia (FD, according to the Rome III Criteria, group IV, n=36) and with recurrent duodenal ulcer (DUD, group V, n=34). Diagnoses were established on the basis of endoscopic imaging and histological examination, 24-hour pH-metry and laboratory tests. Melatonin serum concentration was measured with ELISA method. Blood samples were taken for examination in red-lighted room at 10 p.m. and on the following day at 2 and 6 a.m. The highest concentration of melatonin in all examined groups was determined at 2 a.m. The average melatonin concentration in healthy subjects was  $34,7 \pm 4,8$  pg/ml. In patients with GERD and DUD melatonin concentration was lower than in healthy subjects -  $27,2 \pm 8,5$  pg/ml and  $25,5 \pm 6,2$  pg/ml respectively ( $p < 0,05$ ;  $p < 0,01$ ). The highest concentration of melatonin was found in patients with NERD and FD -  $43,2 \pm 10,8$  pg/ml and  $42,4 \pm 10,1$  pg/ml ( $p < 0,01$ ;  $p < 0,05$ ). The findings of this study support the notion that melatonin exerts beneficial influences on the upper digestive tract. It is likely that high or relatively correct secretion of melatonin is sufficient to prevent peptic changes in esophageal and duodenal mucosa.

**Key words:** *melatonin, nocturnal secretion, gastroprotective properties, functional dyspepsia, duodenal ulcer*

## INTRODUCTION

Even though almost 50 years have passed since the discovery of melatonin, the knowledge on its physiological function is still not complete. It has been established that melatonin influences circadian and seasonal functional rhythm of endocrine glands - hypophysis, thyroid gland, suprarenal glands and gonads (1). Numerous investigations concern the evaluation of melatonin influence on neurological or psychological disturbances and sleeping disorders (2, 3). Particularly, it has been confirmed that a decrease of melatonin secretion in some seasons (autumn - winter) may lead to seasonally related depression (4). Recently, the results of the researches have provided the evidence that melatonin is synthesized not only in the pineal gland, but also in different organs, the special attention has been directed to the digestive tract where total quantity of melatonin is considerably greater than in the pineal gland (5, 6). It was demonstrated that enterochromaffin cells (EC) in the gut are the source of melatonin and have the capability of absorbing and synthesizing indole components (7). These observations were further supported by detection in the gut mucosa enzymes engaged in melatonin synthesis, e.g. hydroxyindole-O-methyltransferase (8) and N-acetyltransferase (9). This is supported by the observation that after pinealectomy melatonin can be detected in the blood of experimental animals (10). Probably, a part of blood melatonin has a source in the digestive system, especially during daytime. At bedtime hours, melatonin is mainly secreted by the pineal gland, but it may also play a considerable role in the alimentary system (11). In particular, its gastroprotective effect is connected with the beneficial influence of the oxidative metabolism in gastric mucosa (12). Melatonin is a powerful direct free radical scavenger as well as an indirect antioxidant (13, 14). It protects gastric mucosa against destructive activity of free radicals during the ischaemic-reperfusion injury process (15) as well as against stress-induced ulcers (16) and due to non-steroidal anti-inflammatory drugs and other gastrototoxic agents (17 - 19). Furthermore, Kato *et al.* (20) in experimental investigations demonstrated inhibitory action of melatonin on secretion of HCl and pepsin. The majority of these observations have been made in experimental studies, in which melatonin was administered in pharmacological doses. Rather few studies on gastrointestinal role of melatonin have been carried out in humans. This promoted us to commence the present investigation. Considering the above observations, we have undertaken our own study protocol on the nocturnal secretion of melatonin in patients with and without peptic changes in the upper part of the gastrointestinal tract.

## MATERIAL AND METHODS

The investigations were carried out in 149 subjects, including 86 women and 63 men, aged 21-46 years (mean age 34.6 yrs). Diagnosis of the disorders of the upper gastrointestinal tract was based on imaging examinations (endoscopy, ultrasound) and laboratory tests, especially those

evaluating the function of liver and pancreas. *H. pylori* infection was also diagnosed by performing the urea breath test - UBT-<sup>13</sup>C (Fanci-2, Fischer Analyzer Instrument, GmbH). Moreover, 24-hour esophageal pH-metry was conducted in 28 patients and CT abdominal scans were obtained in 3 subjects. Based on the above diagnostic results five study groups were distinguished:

- 30 clinically healthy persons constituted group I
- 24 patients with non-erosive reflux disease (NERD) were selected for the study using a standardized questionnaire (group II)

The group II patients had no endoscopic changes in esophageal mucosa, but their results of the 24-hour pH-metry ranged from 19.9 to 96.1 points of DeMeester's Scale (mean value 38.6 points). 11 patients in this group were infected by *H. pylori*.

- Group III consisted of 25 patients with gastroesophageal reflux disease (GERD) grade A and B according to Los Angeles criteria.

• 36 patients with functional dyspepsia constituted group IV. They suffered from chronic or recurrent epigastric pain as a predominant symptom. No macroscopic changes of gastric mucosa were observed in these subjects in on endoscopic evaluation. The patients were enrolled to the study group according to Rome III Criteria (Epigastric Pain Syndrome - EPS). In 17 of them UBT-<sup>13</sup>C test was positive.

- Group V consisted of 34 patients with recurrent duodenal ulcer. 18 of them were *H. pylori* infected.

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

In the clinical assessment, a 10-point scale of symptoms intensity was applied. With scheme the result of 7 - 10 points was considered as severe (subgroup S) and 3 - 6 points as moderate symptoms (subgroup M). Two different subgroups were also distinguished related to *H. pylori* infection. Seven days before the investigation began the patients were recommended an equal diet and asked to cease

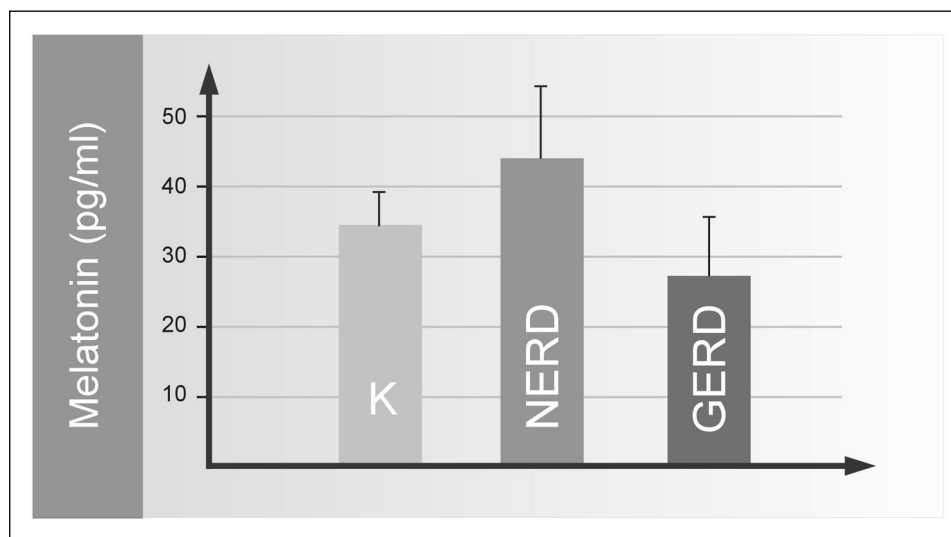


Fig. 1. Mean nocturnal concentrations of serum melatonin in healthy subjects (K) and in patients with non-erosive (NERD) and erosive gastroesophageal reflux disease (GERD); difference between NERD and GERD is significant ( $p<0.01$ )

taking any medication, except from an alkaline drug (Gealcid, Polfa) if it was necessary to reduce epigastric pain or pyrosis. On the day of the study patients remained in a room with red light at nighttime. On this day subjects were given liquid diet (Nutridrink, 4 x 200 ml - 1800 kcal) containing in 100 ml: carbohydrates - 18.4 g, protein - 6.0 g, fat - 5.8 g plus mineral elements and vitamins.

Blood samples were taken for examinations at 10.00 p.m., 2.00 a.m. and 6.00 a.m. After collection, the blood was centrifuged and serum was frozen at the temperature - minus 80°C. Melatonin concentration was measured with ELISA method using the Labsystem Multiscan and antibodies of the Immuno-Biological Laboratories (catalogue RE 54021). Approval of the Bioethical Committee of the Medical University as well as a written consent from each patient enrolled in the study was obtained.

### Statistical analysis

Mann-Whitney U and chi-square tests were used for statistical comparison between groups;  $p < 0.05$  was regarded as statistically significant. Multivariate analysis was performed using the logistic regression model (forward manner with 0.05 as the significance entry level) in order to assess the independent contribution of some patients risk factors. The following risk factors were included: intensity of symptoms and *H. pylori* infection. The estimated  $\beta$ -coefficients and the corresponding P values are given on the basis of the results of this analysis.

## RESULTS

The highest concentration of melatonin was determined in all examined groups at 2.00 a.m. Melatonin levels observed in individual subjects differ considerably at all points of blood sampling. For this reason the mean concentrations at bedtime were used for statistical analysis.

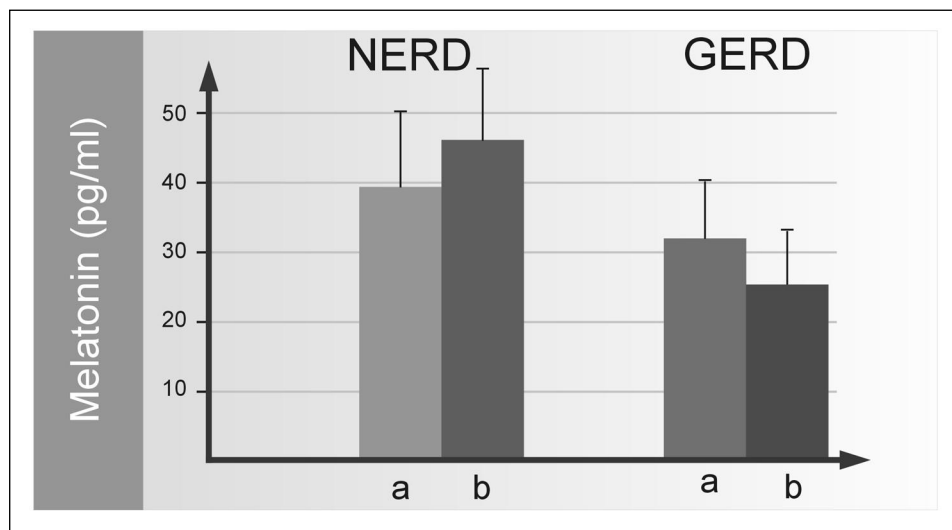


Fig. 2. Mean nocturnal concentrations of serum melatonin in patients with moderate (a) or severe (b) symptoms of gastroesophageal reflux disease; difference between NERD and GERD is not significant ( $p > 0.05$ )

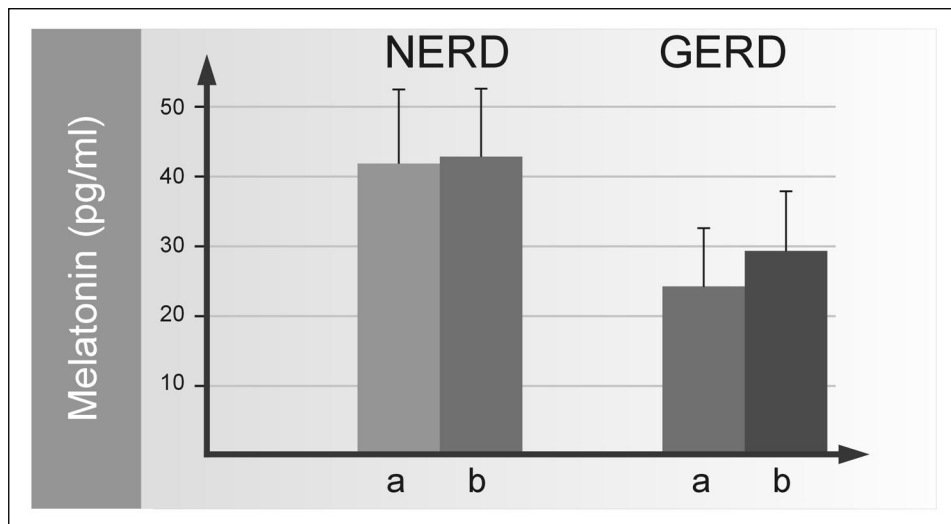


Fig. 3. Mean nocturnal concentrations of serum melatonin in patients with (a) or without (b) infection of *H. pylori*; difference not significant ( $p > 0.05$ )

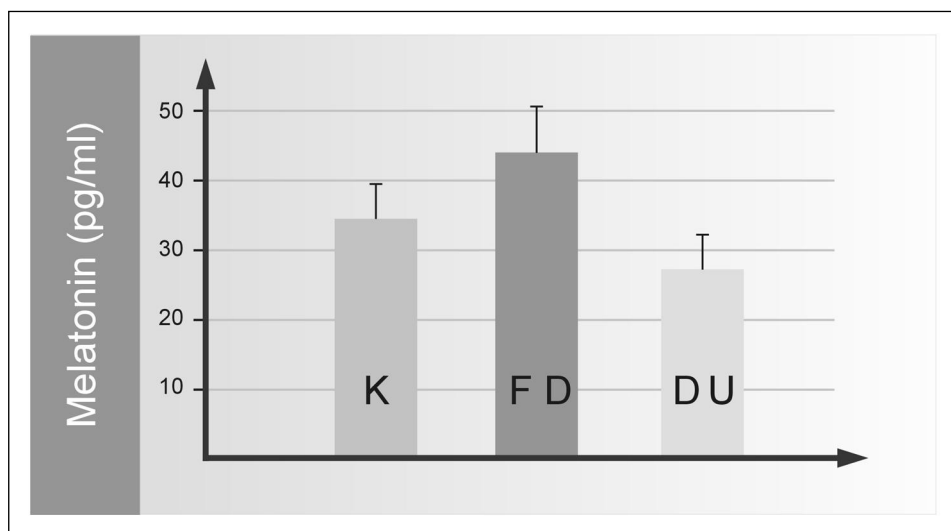


Fig. 4. Mean nocturnal concentrations of serum melatonin in healthy subjects (K) and in patients with functional dyspepsia (FD) and duodenal ulcer (DU); difference between FD and DU is significant ( $p < 0.01$ )

Mean concentration of melatonin in healthy subjects was  $34.7 \pm 4.8$  pg/ml. In patients with NERD melatonin concentration was higher -  $43.2 \pm 10.8$  pg/ml ( $p > 0.05$ ) and in GERD patients lower in than in controls -  $27.2 \pm 8.5$  ( $p > 0.05$ ).

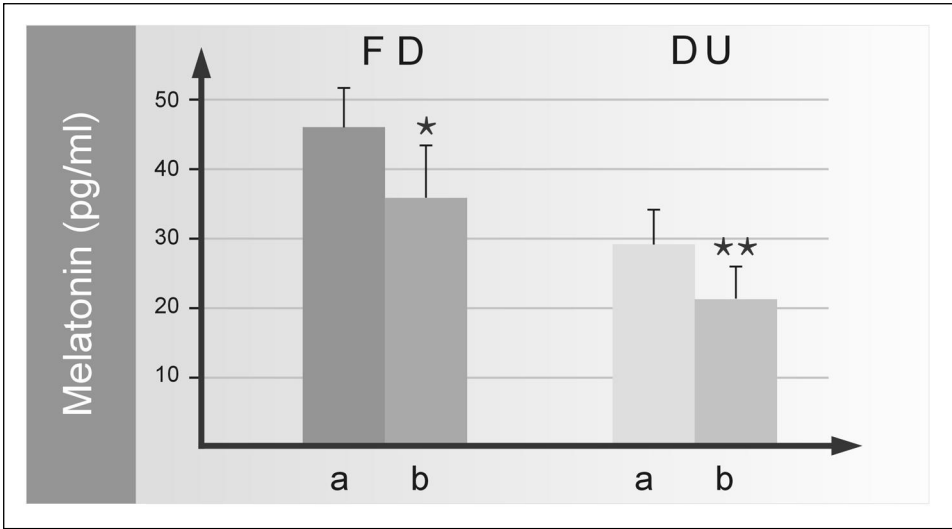


Fig. 5. Mean nocturnal concentrations of serum melatonin in patients with moderate (a) or severe (b) symptoms of functional dyspepsia (FD) and duodenal ulcer (DU);  
\* -  $p < 0,05$  \*\* -  $p < 0,01$

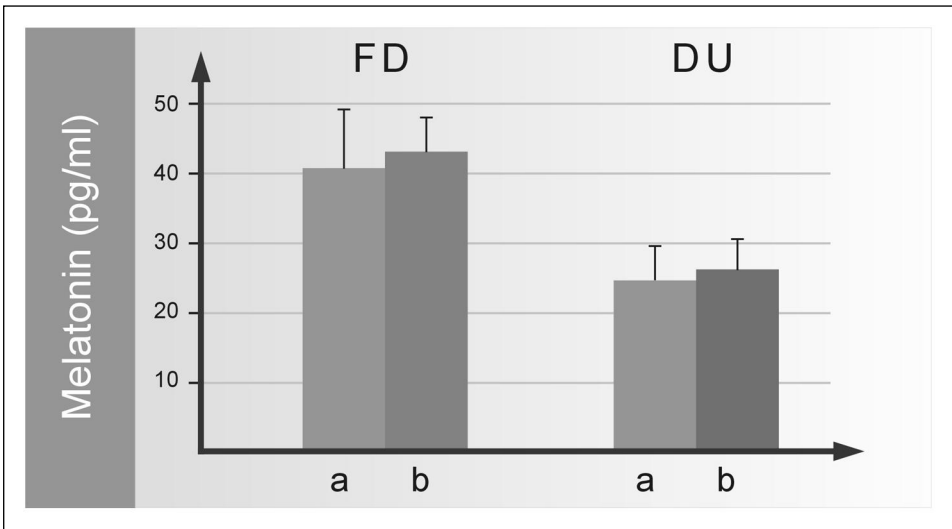


Fig. 6. Mean nocturnal concentrations of serum melatonin in patients with (a) or without (b) infection of *H. pylori*; difference is not significant ( $p > 0.05$ )

The difference between the values in NERD and GERD subjects reached statistical significance ( $p < 0.01$ ) (Fig. 1).

No significant differences were observed between subgroups of patients with GERD distinguished according severity of symptoms: moderate vs severe (Fig. 2).

These values in *H. pylori* infected patients did not differ significantly from those in *H. pylori* negative subjects (*Fig. 3*).

In patients with functional dyspepsia (FD) mean concentration of melatonin was not significantly higher than in the control group ( $p > 0.05$ , *Fig. 4*), while in the group of subjects with duodenal ulcer (DU) lower melatonin concentration was noted but the difference between DU and controls did not reach statistical significance ( $p > 0.05$ ). Difference between mean values in patients with FD and DU was statistically significant ( $p < 0.01$ ).

In patients with severe dyspeptic symptoms, melatonin concentration was significantly lower than in those with moderate complaints -  $36.7 \pm 8.3$  pg/ml and  $46.5 \pm 6.5$  pg/ml, respectively ( $p < 0.05$ , *Fig. 5*). Similar differences related to the severity of symptoms were observed in duodenal ulcer patients -  $29.5 \pm 5.1$  pg/ml and  $21.3 \pm 4.5$  pg/ml ( $p < 0.01$ , *Fig. 5*). No differences were noted between subgroups of patients with or without *H. pylori* infection in both FD and DU subjects (*Fig. 6*).

#### DISCUSSION

In previous investigations we demonstrated low nocturnal secretion of melatonin in duodenal ulcer patients using radioimmunoassay (21). In the present study using immunoenzymatic method (ELISA) we have obtained the similar results in these patients. The studies on melatonin secretion are carried out in different aspects. Among others they try to determine the circadian rhythm, what requires blood sampling minimum every two hours. For the clinical purposes some investigators measure melatonin serum concentration only twice, that is at 9.00 a.m. (light period) and 2.00 a.m. (dark period) and the differences in the day/night patterns are taken for evaluation (22). The interests of gastroenterologists are mainly focused on studies on melatonin nocturnal secretion. In our own investigations melatonin concentration in blood was measured at three points of time: at 10.00 p.m., 2.00 a.m. and 6.00 a.m. The decision of the time of blood sampling was based on our clinical experience. 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. This also proves the complexity of the regulatory mechanisms of the alimentary system.

These observations support the results of numerous experimental studies indicating gastroprotective action of melatonin. The results of the study suggest the possible role of melatonin in pathogenesis of duodenal ulcer; other investigators share the similar opinion. Komarov *et al.* (23, 24) have noted lower secretion of melatonin in both an active phase as well as in remission of duodenal ulcer disease.

Moreover, they revealed that secretion of melatonin is particularly depressed in autumn, when exacerbation of this disease is usually frequent. Malinovskaia *et al.* (25) observed that melatonin in urine is lower in every phase of ulcer patients. Rapoport *et al.* (26) described beneficial changes in the structure of gastric mucosa in duodenal ulcer patients after melatonin administration. In the present study, we have observed lower melatonin concentration in patients with grade A and B erosive oesophagitis. Probably, in more severe changes of oesophageal mucosa (C and D grade of Los Angeles criteria or a peptic ulcer in the lower part of the oesophagus) secretion of melatonin is similarly depressed as in duodenal ulcer patients. It is very important that in subjects with NERD and FD secretion of this hormone remains relatively high. It is not clear why in these patients melatonin secretion is not diminished. Maybe 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. Nevertheless, melatonin seems to play a crucial role in this process. Furthermore a very important property of melatonin is stimulation of bicarbonate secretion in duodenum. This mechanism is particularly important in duodenum where melatonin acts on MT<sub>2</sub> receptors of enterocytes (27). It has been suggested that melatonin stimulates duodeno-pancreatic axis and increases secretion of alkaline pancreatic juice (28). Maybe interdigestive and 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. Probably this compensatory mechanism is sufficient only in healthy subjects, less protective in NERD and FD, and ineffective in patients with GERD and DU. These suggestions need confirmation in the next clinical study.

#### CONCLUSIONS

1. The findings of this study show that melatonin exerts beneficial influences on the upper digestive tract.
2. It is likely that high or relatively correct secretion of melatonin is sufficient to prevent peptic changes in oesophageal and duodenal mucosa.

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