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## IS MELATONIN INVOLVED IN THE IRRITABLE BOWEL SYNDROME?

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There is a substantial evidence that large quantities of melatonin are produced in gastrointestinal tract, however, is still unclear which is the role of melatonin in digestive system in human physiology and pathophysiology. In the present study we investigated urinary excretion of a main melatonin metabolite, 6-sulphatoxymelatonin, in patients with irritable bowel syndrome (IBS). The investigation was carried out in 67 persons, both sexes, aged 20-45 years old who according to Rome III Criteria were diagnosed as sufferers of constipation (C-IBS, n=21 persons) or diarrhoea (D-IBS, n=24 persons) form of irritable bowel syndrome and as healthy subjects (K, n=22), matched for control. Samples were obtained from the collected diurnal urine. The concentration of 6-sulphatoxymelatonin (6-SMLT) was measured with ELISA method, creatinine (crea) was automatically analyzed with biochemical analyzer and 6-SMLT/crea calculated. There were statistically significant differences between groups: the 6-SMLT/crea level was lower in C-IBS (103.86±82.83 ng/mg) and D-IBS (112.72±85.29 ng/mg) groups compared to K group (202.7±89.28 ng/mg), respectively, p=0.002, p=0.003. There were no differences between C-IBS and D-IBS groups, however, there were observed differences between men and women with C-IBS. The 6-SMLT/crea. level was higher in women with C-IBS (139.31±96.45) compared to men with C-IBS (35.51±41.05) (p=0.04). These results suggest that different melatonin secretion and metabolism may be involved in the pathogenesis of irritable bowel syndrome.

Key words: *melatonin, 6-sulphatoxymelatonin, irritable bowel syndrome*

### INTRODUCTION

Functional disorders, among them irritable bowel syndrome (IBS), are very common and troublesome gastroenterological problems. IBS affects up to one fourth of general population at some time of their lives and has been shown to account for between 40 and 70% of the gastroenterologists workload. Melatonin (5-methoxy-N-acetyltryptamine), the molecule extracted from the bovine pineals almost fifty years ago by Lerner *et al.* (1), occurs in a wide range of organisms as different as herbs, fungi, bacteria, algae, and mammals (2-4). It acts as a free radical scavenger and indirect antioxidant. Today melatonin is known as a major regulator of the 'internal clock', biological modulator of mood, sleep, retinal physiology and immunological function. In animals melatonin is the main factor of seasonal-reproductive physiology and sexual behavior (5). The gastrointestinal tract of vertebrates is a rich source of extrapineal melatonin and the concentration of melatonin in the gastrointestinal tissues surpasses blood levels by 10-100 times. The digestive tract contributes significantly to melatonin concentrations in the peripheral blood, particularly during the day. Melatonin is thought to be produced in the GI mucosa in serotonin-rich enteroendocrine cells (EEC) from which it can be released postprandially (2, 3, 6). Indeed, the release of gastrointestinal melatonin appears to be related to periodicity of food intake (3, 5, 6). However, the current knowledge on physiological function in the gastrointestinal tract is still poor. It has been established that melatonin influences circadian and seasonal rhythmicity, exerts an antioxidant activity. According to

some recent studies, melatonin exhibits also immunomodulatory and anti-inflammatory activities.(7-11). Clinically, melatonin is considered to have a potential for a prevention as well as the treatment of peptic ulcer, colorectal cancer, ulcerative colitis, acute pancreatitis, children colic and diarrhea and some functional disorders of gastrointestinal tract, mainly functional dyspepsia (FD) and IBS (8-10, 12, 13).

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder affecting up to 3-15% of the general population. It is characterized by unexplained abdominal pain, discomfort and bloating. The pathophysiology of IBS is considered to be multifactorial, involving disturbances of the brain-gut-axis. IBS has been associated with abnormal gastrointestinal motor functions, visceral hypersensitivity, psychosocial factors, autonomic dysfunction and mucosal inflammation (8, 11). There are some hypotheses concerning the role of melatonin in pathogenesis of IBS (9, 12, 13). To get further insight into the problem we decided to analyze the excretion of main melatonin metabolite 6-sulphatoxymelatonin in patients with (IBS).

### MATERIALS AND METHODS

The investigation was carried out in 67 persons, both sexes, aged from 20 to 45 years old. According to Rome Criteria III, 21 patients (31.34%) were diagnosed as a constipation (C-IBS, 11 women - 52.38 %, 10 men - 47.62%) and 24 (36.82%) as a diarrhea (D-IBS, 12 women - 50%, 12 men - 50%) form of

irritable bowel syndrome. The control group (K) constituted of 22 (32.84%) healthy subjects (10 women - 45.45%, 12 men - 54.54%). The Bioethical Committee of the Medical University of Lublin gave their consent to the study. We excluded from the study patients with renal failure (creatinine >1.4 mg/dl), liver failure, depression, likewise patients using beta-blockers, steroids, benzodiazepines, selective serotonin reuptake inhibitors. Control group consisted of healthy volunteers.

The urine was collected over 24-hours period sampling. The total urine volume was recorded and aliquots were frozen at -20°C. Measurement of 6-sulphatoxymelatonin (6-SMLT) was done by using highly specific enzyme linked immunoassay (ELISA IBL Hamburg Germany - RE 54031). Urine creatinine was measured with the standard biochemical analyzer. The results were expressed as the ratio of the amount of 6-sulphatoxymelatonin in ng/dl urine divided by the amount of creatinine (6-SMLT/crea) to compensate for differences in urine volume.

### Statistics

The data are presented as means with standard deviation. The range of values is also given. For statistical analysis one way Anova and Shapiro-Wilk Levine's test were used. P values of <0.05 were considered statistically significant.

## RESULTS

As shown in Fig. 1, the urinary excretion of 6-SMLT/crea (ng/mg) in healthy controls was significantly higher:  $202.7 \pm 89.3$  ng/mg than those in the group with constipation (C-IBS):  $103.9 \pm 82.8$  ng/mg and diarrhea (D-IBS):  $112.7 \pm 85.3$  ng/mg ( $F=8.97$ ,  $P=0.0004$ ). Further analysis confirms high statistical significance between these groups as  $p=0.002$  for K vs C-IBS and  $P=0.003$  for K vs. D-IBS. There was no difference between C-IBS and D-IBS groups ( $P=0.94$ ).

Significantly higher level of 6-SMLT/crea was observed in healthy women when compared to healthy men ( $F=8.49$ ,  $P=0.009$ ). Similarly, in C-IBS group, women expressed significantly higher level of 6-SMLT/crea as compared to men ( $F=5.10$ ,  $P=0.04$ ). There were no statistical differences between men and women in diarrhoea group (D-IBS) (Table 1). Women from the control group excreted statistically significant higher level of 6-SMLT/crea (ng/mg) with  $P=0.03$  versus those with constipation (C-IBS) and  $P=0.02$  versus diarrhea (D-IBS) group. There were no differences in 6-SMLT/crea (ng/mg) level in women with D-IBS compared to those from C-IBS group (Fig. 2). Men from the control group had significantly higher 6-SMLT/crea (ng/mg) level compared to men from C-IBS ( $p=0.006$ ) and from D-IBS group ( $p=0.04$ ). There were no differences in 6-SMLT/crea (ng/mg) level in men with D-IBS compared to those from C-IBS group (Fig. 3).

## DISCUSSION

Irritable bowel syndrome is a frequent disease of unknown ethiopathogenesis. The Rome Criteria III help to diagnose IBS in patients with symptoms from gastrointestinal tract (15). Diagnostic criteria for IBS include recurrent abdominal pain or discomfort at least 3 days per month in the last 3 months associated with 2 or more of the following: improvement with defecation, onset associated with a change in stool frequency or onset associated with a change in form (appearance) of stool.

Melatonin is involved in the regulation of gastrointestinal motility and sensation and when administered orally in

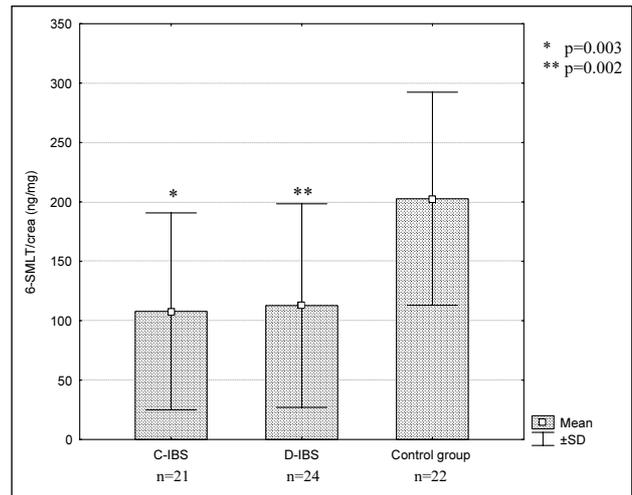


Fig. 1. The 6-sulphatoxymelatonin/creatinine (SMLT/crea) (ng/mg) excretion in healthy controls and patients with irritable bowel syndrome (IBS) with constipation (C-IBS) and diarrhea (D-IBS).

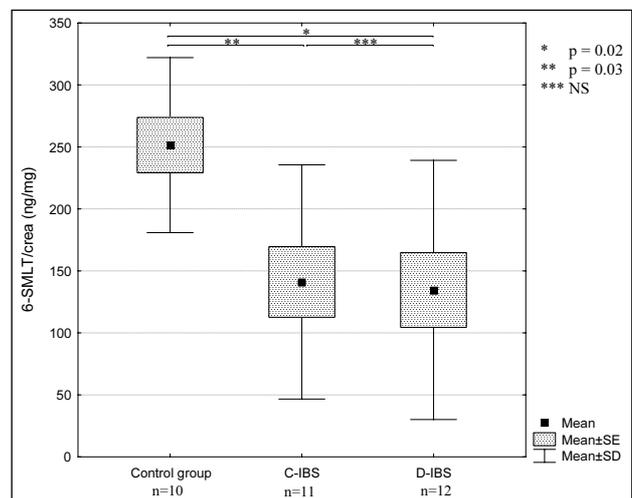


Fig. 2. The 6-sulphatoxymelatonin/creatinine (SMLT/crea) (ng/mg) excretion in females with different forms of irritable bowel syndrome (IBS)

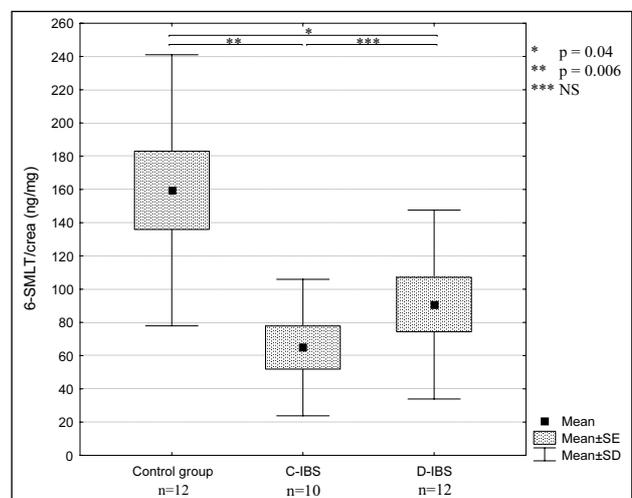


Fig. 3. The 6-sulphatoxymelatonin/creatinine (6-SLMT/crea) (ng/mg) excretion in male groups with different forms of irritable bowel syndrome (IBS)

Table 1. Urinary excretion of 6-sulphatoxymelatonin/creatinine (ng/mg) in patients with irritable bowel syndrome (IBS): both form: IBS-Constipation (C-IBS) and IBS-Diarrhea ( D-IBS), and healthy subjects.

Groups of IBS patients	6- sulphatoxymelatonin/creatinine level (ng/mg)					
	Women			Men		
	Mean	Range	Standard deviation	Mean	Range	Standard deviation
IBS-Constipation	139.31	74.51-204.10	96.45	64.88	35.51-94.24	41.05
IBS-Diarrhea	134.68	68.27-201.10	104.53	90.75	54.64-126.86	56.83
Healthy subjects	255.04	204.85-305.22	70.15	159.40	107.57-211.23	81.57

pharmacological doses it had the beneficial effect on abdominal pain in IBS patients without improving the sleep disturbances (16). It was also shown that oral melatonin significantly increased colonic transit time in healthy subjects, and may be a promising candidate for the future research of agents that can modulate bowel motility (17). Melatonin synthesized in the enteroendocrine cells of the intestinal mucosa reaches the liver *via* the portal vein (6). Melatonin is a potent stimulant of duodenal mucosal bicarbonate secretion which neutralizes the acid content of stomach in the duodenum and also seems to be involved in the acid-induced stimulation of the secretion (7, 18). Melatonin protects the gastrointestinal mucosa due to an antioxidant action, reduction of secretion of hydrochloric acid, the stimulation of the immune system, fostering epithelial regeneration, and increasing microcirculation (3, 7, 8).

The current study showed that patients with IBS had significantly lower 6-SMLT/crea level compared with healthy controls. The lack of statistical difference in 6-SMLT/crea levels between the constipation and diarrhoea groups is difficult to explain. In some patients the symptoms could be recurrent or there could be some subjects with mixed (IBS-M) or unsubtyped (IBS-U) IBS. Most studies have shown a female predominance in patients with IBS (12, 14, 15, 19, 20). Our results agree with the results obtained by Lu *et al.* who performed the study on female patients with IBS and found decreased salivary melatonin and urine 6-SMLT level compared to non-IBS volunteers (20). Low melatonin levels were observed in women with eating disorders, moreover, the low melatonin concentrations were related to increased depressive symptomatology especially sadness, bodily discomfort, inner tension, difficulties in attention concentration and pain (19, 20). In the current study we observed the lower level of 6-SMLT/crea in men compared to women and the issue deserves further investigations, although some differences concerning the function of serotonin and its receptors in male and female patients with IBS are known. Serotonin, an endogenous amine and the precursor of melatonin, synthesized and released from EEC of the gastrointestinal mucosa is thought to play an important role in the pathogenesis of IBS (12). Antagonists of serotonin 5-HT<sub>3</sub> receptor are beneficial in patients with diarrhea-form of IBS, whereas partial agonist of serotonin 5-HT<sub>4</sub> receptor (tegaserod) alleviates symptoms of IBS with constipation, especially in females (12, 21).

The role of melatonin as regulator of circadian and seasonal rhythmicity has been established (3, 8, 10). Patients with functional disorders of the gastrointestinal tract had also sleep disorders and some of them suffer from the increased neural excitability and anxiety (10, 22). There were speculations concerning a possible role of melatonin in functional dyspepsia (FD), particularly ulcer-like dyspepsia. In two types of FD: one with epigastric pain (EPS) and second with postprandial distress syndrome (PDS), the melatonin level is varied and different dyspeptic symptoms maybe related to differences in melatonin secretion (10). In patients with IBS, complaints of poor sleep are common; self reported sleep

disturbance can be regarded as one of the most important extraintestinal symptoms of IBS, which markedly affects quality of life and psychosocial well being (14). In double blind placebo controlled study, Songh *et al.* have shown, that melatonin improves abdominal pain in IBS patients having sleep disturbances (13). Current conventional treatment for irritable bowel syndrome is far from satisfactory. Despite multiple therapeutic interventions no long-term effect is achieved. On the other hand, hypnotherapy has been shown to be very effective in the treatment of IBS with up to 80% of patients showing an improvement of their symptoms (22). These observations emphasize the possible role of melatonin in pathogenesis of irritable bowel syndrome and in the therapy (23, 24).

Our results also suggest that disturbances in melatonin metabolism and secretion may be involved in pathogenesis of different gastrointestinal diseases especially those coexisting with psychoemotional disorders.

Conflict of interest statement: None declared.

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Received: October 10, 2008

Accepted: December 6, 2008

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