JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2010, 61, 3, 295-300
www.jpp.krakow.pl

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EVALUATION OF URINARY 6-HYDROXYMELATONIN SULPHATE EXCRETION IN WOMEN AT DIFFERENT AGE WITH IRRITABLE BOWEL SYNDROME

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Melatonin (MT) exerts a beneficial action in the treatment of many diseases, among them also in irritable bowel syndrome (IBS). Its secretion decreases with age, particularly, in the postmenopausal period in women. It has not been determined whether these changes can have an impact on the clinical picture of IBS. The study aimed at evaluating the urinary excretion of the main MT metabolite - 6-hydroxymelatonin sulphate (6-HMS) in women at different age with IBS. The investigations were carried out in five groups of 30 women each. Group Ia (the controls) – premenopausal healthy women (20-39 years), group Ib (the controls) – postmenopausal healthy women (46-66 years), group II – women with constipation predominant IBS (IBS-C; 19-42 years), group III – women with diarrhoea predominant IBS (IBS-D; 20-39 years), group IV- women with IBS-C (49-68 years), group V – women with IBS-D (48-69 years). The diagnosis of IBS was based on the Rome III Criteria after excluding other diseases. On the day of the study the patients remained on the same liquid diet (Nutridrink – 3x400 ml) and 1500 ml of still mineral water. 6-HMS concentration in urine was measured by ELISA method applying IBL antibodies (RE-54031, Immunological Laboratories). The results showed that 24-hour urinary 6-HMS excretion in the studied premenopausal women were as follows: group Ia – 15.13±5.83 μg/24 h, group II – 28.85±12.59 μg/24 h ($p<0,01$), group III – 26.10±11.76 μg/24 h ($p<0,01$) and in the postmenopausal subjects they were: group Ib – 10.66±3.23 μg/24 h, group IV – 13.73±5.09 μg/24 h (($p=0,02$), group V – 21.39±10.88 μg/24 h ($p<0,01$). In women with IBS-C the obtained results of 24-hour 6-HMS urinary excretion were independent on the intensity of clinical symptoms. On the other hand, in women with IBS-D, both in the group III and V, higher intensity of ailments was accompanied by significantly increased 6-HMS urinary excretion. The results of the study allowed drawing the following conclusions: (1). 24-hour 6-HMS urinary excretion in women with the constipation-predominant (IBS-C) as well as the diarrhoea-predominant IBS (IBS-D) is higher than in healthy persons both in the premenopausal and postmenopausal period. (2). Relatively high 6-HMS urinary excretion in postmenopausal women with IBS-D indicates an adaptive increase in MT secretion from EC in the gut.

Key words: *melatonin, 6-hydroxymelatonin sulphate, irritable bowel syndrome, pre- and postmenopausal women, tumor necrosis factor alpha, NF kappa B, interleukin 6*

INTRODUCTION

Irritable bowel syndrome (IBS) is the most common functional disease of the gastrointestinal tract of the complex and not fully understood pathogenesis. Among various etiopathogenic factors there are: genetic, environmental and psychoemotional (1). In the International Classification of the Diseases (ICD-10) IBS is mentioned twice, both in the chapter about the gastrointestinal diseases (K 58) and in the part about mental and behavioral disorders as vegetative changes in the form of somatization (F 45). From the physiological point such a classification is not a contradiction as the brain-gut axis form a functional unity. Both, in CNS and in the alimentary system, a number of the same receptors, neurotransmitters and hormones are present, which play a role in the functional regulation of these systems. Serotonin plays a crucial role among the neurotransmitters. Its major sources are nervous cells but also numerous enterochromaffin cells (EC) localized in the gastrointestinal tract (GIT) (2). EC also produce MT (3). For both of these enterohormones L-tryptophan (L-Trp) is a precursor.

The physiological role of serotonin in the alimentary tract is quite well understood. It plays a regulatory role in the motor and secretory function of the gastrointestinal tract as well as transmits the functional stimuli from the GIT to CNS (4-6). MT plays equally important role independently on its source, whether it is secreted by the pineal gland or EC. MT belongs to the most potent endogenous anti-oxidant compounds (6, 7). It prevents vascular changes engaged in the process of atherosclerosis by decreasing homocysteine concentration in blood, the level of oxidative stress and increasing NO production (8). Due to its antioxidant properties and COX-PGE_2 system activation, it exerts protective action on the gastrointestinal mucosa (9-11). MT also acts as a smooth muscle relaxant in the

alimentary system and, in this respect, it is an antagonist to serotonin (12). It can be supposed that disturbances of MT homeostasis, similarly to these of serotonin, can play a role in the pathogenesis of IBS. This hypothesis is confirmed by our own clinical observations, which show that MT exerts a beneficial effect in the constipation predominant irritable bowel syndrome (IBS-C).

MT secretion from the pineal gland varies in different year seasons and in most of people it is decreased in autumn and winter. It is accompanied by mood depression but also by exacerbation of various ailments, especially these of the alimentary tract (13). Gastrointestinal functional diseases are also characterized by chronic course with the periods of relapses and remissions what perhaps partly depends on seasonal rhythm of MT secretion from the pineal gland (14, 15). Such correlation has not been found in young people. In the elderly, both seasonal and daily rhythms of MT secretion vanish and its secretion clearly decreases. This period from the physiological point of view is called melatonin-pause (16). It is not known whether these changes can have their impact on the clinical picture of IBS. These doubts inspired us to conduct our own studies in this field.

IBS is 3-fold more prevalent in women than in men, and in the postmenopausal women it occurs even with 6-fold higher prevalence. It results from the involutional changes of gonadal function but the role of melatonin in this process is also very probable. Okatani *et al.* (17) showed that in the postmenopausal period in women there is a consistent drop in MT secretion. Vakkuri *et al*. (18) observed that a significant decrease in MT secretion is preceded by the rise in FSH concentration. Moreover, they found the positive correlation between MT concentration in urine and FSH serum concentration. According to these authors, in women at the age of 40-44 years MT concentration in urine is 41% lower than in women of 30-34 years. Similarly, a further decrease of 33% of MT concentration in urine was found in women at the age of 55-59 years compared to these at the age of 50-54 years. In the studies mentioned above MT concentration in urine was evaluated. However, urinary excretion of unmetabolized MT is low and with the normal liver function it does not exceed 1% of the total pool. Because of these reasons this parameter is not the best index of secretory activities of pinealocytes and EC. In many authors' opinion, far more useful for this purpose is the measurement of MT metabolites urinary excretion (19). The major metabolite of MT is 6-hydroxymelatonin, which in 90% is conjugated with sulphate and in 10% with glucuronate in liver. These metabolites are excreted mainly in urine but also partly in bile. It has been recognized that the amount of 6-hydroxymelatonin sulphate (6-HMS) excreted in urine is a good index of MT synthesis and excretion (20). Moreover, 6-HMS also exerts antioxidative properties and its appropriate level can reduce some of the unfavourable consequences of oxidative metabolism disturbances.

Our study aimed at evaluating 6-hydroxymelatonin sulphate (6-HMS) excretion in women with IBS considering their age.

MATERIAL AND METHODS

The investigations were carried out in 180 women, aged 19-69 years. Six groups were distinguished of 30 persons each.

Group Ia (controls) – clinically healthy women with sporadic ailments of the gastrointestinal tract aged 20-39 years (mean age 28.4 years).

Group Ib (controls) – clinically healthy women aged 46-66 years (mean age 56.2 years).

Group II – women with constipation predominant IBS (IBS-C) aged 19-42 years (mean age 31.0).

Group III – women with diarrhoea predominant IBS (IBS-D) aged 20-39 years (mean age 29.3).

Group IV – women with constipation predominant IBS (IBS-C) aged 49-68 years (mean age 56.9).

Group V – women with diarrhoea predominant IBS (IBS-D), aged 48-69 years (mean age 58.4).

Patients from the groups IV and V were in the postmenopausal period. The time that had passed since the last menstruation varied from 2 to 18 years and the mean FSH concentration was 93.4 IU/l. The diagnosis of IBS was based on the Rome III Criteria. In all the subjects enrolled in the study, the endoscopy examinations of the upper and lower part of the gastrointestinal tract were performed, histopathology of the colon mucosa was obtained, abdominal ultrasound was performed as well as the laboratory tests: the blood cell count, CRP, glucose, electrolytes, bilirubin, urea, creatinine, cholesterol, triglycerides, TSH and activity of the following enzymes: AST, ALT, GGTP, FA, amylase and lipase.

In patients with IBS-D, additionally, parasitological and bacteriological stool examinations, biopsy and histopathological analysis of the small bowel mucosa were performed. Patients with organic, metabolic and psychiatric diseases as well as on any long-standing pharmacological treatment and cigarette smokers were excluded from the study.

Applying 10-point scale of the symptoms intensity in both groups, the patients with moderate (1-5 points) and exacerbated (6-10 points) symptoms were distinguished. The subgroups were comparable counting from 14 to 16 women each. Seven days prior the evaluations all the medications were withdrawn and the same diet was recommended in all the patients, particularly with a similar daily amount of products reach in L-tryptophan. On the day of the study the subjects remained in the room with only red light in the hours from 9.00 p.m. till 7.00 a.m. and the same liquid diet was administered (Nutridrinks, Nutricia) in the amount of 3x400 ml of the caloric value - 1800 kcal and 1500 ml of the isotonic still water. At the same time the 24-hour urine collection was performed. Urine was kept at +4°C. Immediately after the end of 24-hour urine collection, the volume of urine was measured, centrifuged and the samples were frozen at -70°C. 6-HMS concentration in urine was measured by ELISA method applying IBL antibodies (RE-54031, Immunological Laboratories) and Expert 99 MicroWin 2000 reader (Biogenet). The results obtained were converted from ng/ml to µg/24 h. The study was approved by the Bioethical Committee of Medical University of Lodz (number RNN 238/05/KB) and all the patients enrolled signed the written consent. The data were analyzed statistically applying the Kruskal-Wallis and Mann-Whitney tests and the Statistica-Microsoft Co software.

RESULTS

24-hour urinary 6-HMS excretion in the controls, that is in young and clinically healthy women (group Ia) was 15.13±5.83 µg/24 h. In the similar age groups of patients with IBS, the results differed significantly and were as follows: in IBS-C – 28.65±12.59 µg/24 h, ($p<0.001$) and in IBS-D – 26.10±11.76 µg/24 h, ($p<0.001$) (*Fig. 1* and *Fig. 3*).

In older women in the postmenopausal period, the results showed statistically significant differences too compared with the control group and they were respectively in the controls (group Ib) 10.66±3.23 µg/24, IBC-C – 13.73±5.09 µg/24 h, ($p=0.02$) and IBS-D – 21.39±10.88 µg/24 h, ($p<0,01$) (*Fig. 2* and *Fig. 3*).

In patients with IBS-C the obtained results of 24-hour 6-HMS urinary excretion were independent on clinical symptoms (*Fig. 4*). On the other hand, in women with diarrhea predominant IBS (IBS-D) highly intensified aliments were accompanied by increased 6-

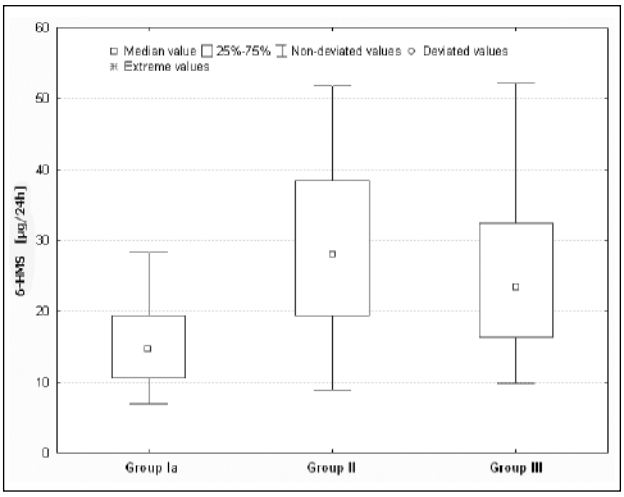

Fig.1. 24-hour 6-HMS urinary excretion in the controls and in the premenopausal women with IBS-C and IBS-D; Group Ia – controls; Group II – premenopausal women with IBS-C; Group III – premenopausal women with IBS-D.

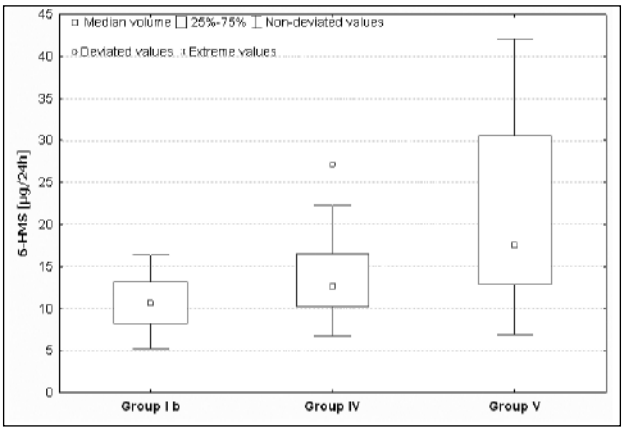

Fig. 2. 24-hour 6-HMS urinary excretion in the controls and in the postmenopausal women with IBS-C and IBS-D. Group I b – controls; Group IV – postmenopausal women with IBS-C; Group V – postmenopausal women with IBS-D.

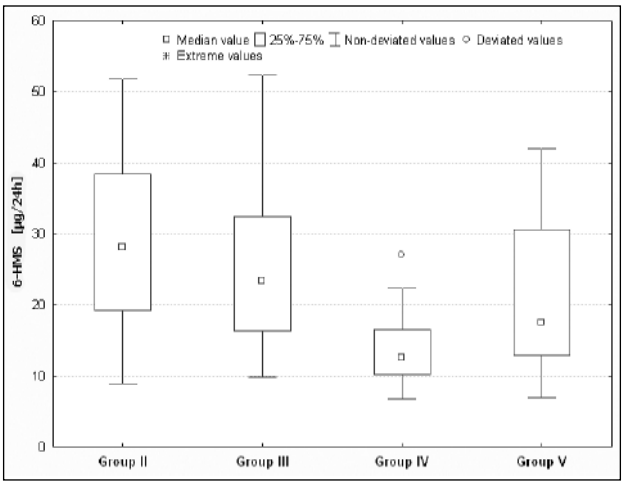

Fig. 3. 24-hour 6-HMS urinary excretion in the pre- and postmenopausal women with IBS-C and IBS-D. Group II – premenopausal women with IBS-C; Group IV – postmenopausal women with IBS-C; Group III – premenopausal women with IBS-D; Group V – postmenopausal women with IBS-D.

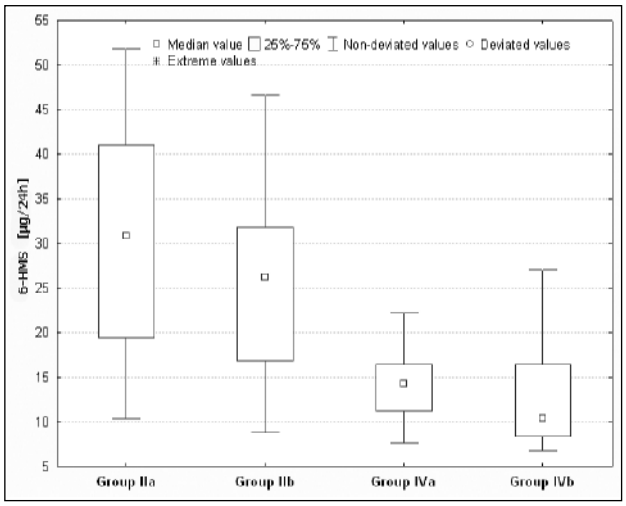

Fig. 4. 24-hour 6-HMS urinary excretion according to symptom severity in pre- and postmenopausal women with IBS-C. Group IIa – premenopausal women with moderate symptoms intensity of IBS-C; Group IIb – premenopausal women with exacerbated symptoms intensity of IBS-C; Group IVa – postmenopausal women with moderate symptoms intensity of IBS-C; Group IVb – postmenopausal women with exacerbated symptoms intensity of IBS-C.

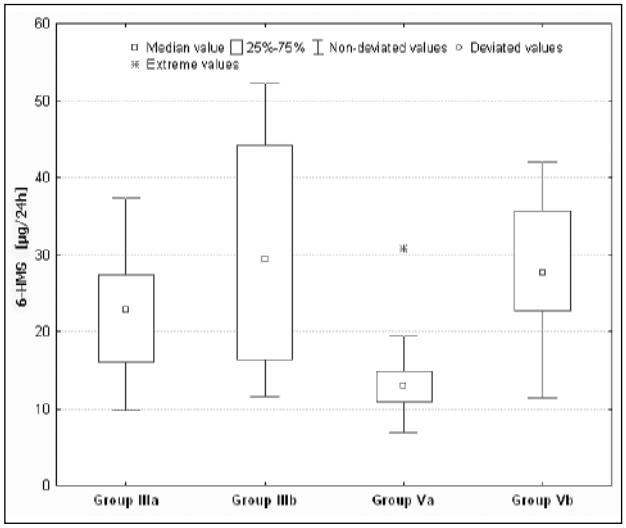

Fig. 5. 24-hour 6-HMS urinary excretion according to symptom severity in pre- and postmenopausal women with IBS-D. Group IIIa – premenopausal women with moderate symptoms intensity of IBS-D; Group IIIb – premenopausal women with exacerbated symptoms intensity of IBS-D; Group Va – postmenopausal women with moderate symptoms intensity of IBS-D; Group Vb – postmenopausal women with exacerbated symptoms intensity of IBS-D.

HMS urinary excretion and they were respectively in the group III – 22.51±8.66 and 29.70±13.56 µg/24 h, ($p<0.05$) and in the group V – 13.70±5.80 and 28.11±9.82 µg/24 h, ($p<0.001$) (*Fig. 5*).

DISCUSSION

Evaluation of MT secretion in humans is extremely difficult because this process is modulated by multiple factors, among others, it is subordinated to seasonal and daily rhythms. It also

significantly changes with age. The most common way to evaluate the full profile of MT secretion within different periods is to measure its serum concentration every several tens of minutes in twenty-four hours. This way of evaluation MT secretion is troublesome for the studied subjects as it disturbs "the physiologic peace" especially at night, which can of course have an impact on the obtained results. Because of these reasons, some authors propose to measure the serum concentration twice at 2.00 a.m. and 9.00 a.m. (21) to determine the day and night activity of the cells secreting MT. Nevertheless, also in such a case the results have only limited value. It is well recognized that during the day with the access of natural white light MT is mainly secreted in the GI system by EC as a result of many stimuli, among them also food intake (9, 22). Single measurement of MT serum concentration at fasting at 9.00 a.m. does not reflect the changes in the synthesis of this hormone during the whole day. In reality, the oscillation of MT concentration in peripheral blood is small. In experimental models in pinealectomized animals, there is no increase in MT night secretion. Despite this, its concentration during the whole day remains relatively constant and is equal to MT concentration before the pinealectomy (23).

There are some differences between pineal and GIT-derived MT. The hormone secreted from the pineal gland exerts endocrine action, whereas this derived from the alimentary tract exerts endocrine, paracrine and luminal actions (22, 24). After entering the portal vain passes through the liver where it is metabolized in 90% and a part of it also enters bile and urine (25). MT may be also metabolized to other products in peripheral tissues when it functions as a scavenger. It can react directly with reactive oxygen species (ROS) and nitrogen species (RNS) giving various derivatives, *e.g.* kynurenine and nitroderivatives (26). The amount of MT derived from the alimentary tract and present in the systemic circulation is small, and on this basis, the conclusions about the total body pool of melatonin cannot be drawn. It is suggested that the evaluation of MT metabolites excretion in urine is a more useful method for this purpose.

The separate problem is the time and the cause-effect dependence between MT and sex hormones. It is well known that MT secretion changes with aging. It can indirectly affect gonadal activity *via* the modification of gonadoliberin and gonadotropins secretion (27). The direct effect of MT on gonadal activity cannot also be excluded. Higher MT concentration in the ovarian vesicles fluid suggests that this hormone may as well be synthesized in gonads (28). These suggestions are supported by the studies confirming the presence of precursor compounds (tryptophan and serotonin) as well as enzymes engaged in MT synthesis (serotonin N-acetyltransferase and hydroxyindole-O-methyl transferase) in the human ovaries homogenates (29). The effect of MT on both synthesis and excretion of sex hormones by ovarian vesicles is not unequivocally stated. Some authors described its inhibitory action on progesterone secretion (30), while others, in contrary, observed MT stimulatory effect (31). At the same time, it was stated that MT did not show any significant impact on estradiol secretion; moreover, it can even inhibit this process (32, 33). The authors mentioned in the introduction, Okatani *et al*. (17) observed the negative correlation between estradiol and MT concentration in postmenopausal women. They also showed that estradiol administration in postmenopausal women reduced nocturnal synthesis of MT.

On the other hand, Luboshitzky *et al*. (32) observed that estrogen treatment in women with hyperandregonemia reduces usually elevated 6-HMS concentration in urine. It is supposed that the association between estrogens and MT is not exclusively based on the feed-back mechanism but it is far more complex with the tropic hormones playing the role in this process (33).

Irrespective of above pathogenic associations, the results of our studies showed that MT secretion in women with IBS decreased after menopause. It justifies the usefulness of MT administration in the treatment of these patients. In IBS MT can also exert a beneficial effect because of its myorelexative properties upon smooth muscles of the gut. It also exhibits regulatory action on the gastrointestinal motor function. This effect can be caused by direct stimulation of the specific receptors (34, 35) regulating the calcium and potassium channels activity as well as indirectly acting on the nervous system (36). This hormone blocs the nicotinic acetylcholine receptors on the nerve endings of the submucosal plexi (37) and activates afferent vagal fibres *via* an increased release of CCK and CCK1R/CCK2R receptors activation (38). Bubenik (22, 39) as well as Harlow and Weekly (40) showed that MT myorelaxative effect is directly proportional to the basic spontaneous tone and amplitude of intestinal contractions. In another study Drago *et al*. (41) observed that MT in the low doses accelerated and in the high doses slowed the intestinal transit in the experimental models in animals. Bubenik (22) described that an increase of GIT MT decreases the speed of food transit time, which may give the body more time to utilize all available food resources.

The clinical studies also proved the efficacy of MT in the treatment of IBS. Song *et al*. (42) recommended the single dose of melatonin of 3 mg in the evening for two weeks achieving abdominal pains resolving, especially in patients with sleep disorders and dystymia. Lu *et al*. (43) also recommended the dose of 3 mg of MT for 8 weeks in patients with IBS and they also achieved the improvement of both somatic and psychiatric state. Saha *et al*. (44) achieved the beneficial effect of 3 mg of MT in IBS after 48 weeks in the follow-up evaluation. These observations encourage conducting further studies to use MT in the treatment of various pathologic states of the alimentary system. Particularly interesting are the observations about anti-inflammatory action of MT, among others, inhibiting gene expression for TNF-α and adhesion molecules as well as stimulating the synthesis of anti-inflammatory cytokines – IL-2, IL-6, IL-12 (45, 46). Konturek *et a*l (46). showed that MT exerted strong anti-inflammatory effects due to inhibition of NFkappaB and TNF-α expression. Due to this action MT and its precursor L-Trp significantly accelerated healing of gastric ulcer in the experimental model in rats.

Although IBS is considered to be a functional disease, the inflammatory factors are also taken into consideration in its pathogenesis. Among others, mastocytes and EC hyperplasia in the intestinal mucosa of the colon in the IBS patients was observed (47) similarly to the mucosal changes in IBD (48). As a consequence of this serotonin considered to be on of the major inflammatory mediators, secretion in the alimentary tract rises. Probably MT secretion increases simultaneously and thanks to it the degree of inflammatory reactions is attenuated.

The results of our studies seem to confirm these suggestions because 6-HMS urinary excretion in women with IBS, particularly these with diarrhoea predominant IBS (IBS-D), was relatively high, also after 48 years. It can be supposed that EC preserve their secretional function far longer than pinealocytes. In this respect, the alimentary tract in the elderly patients takes over the pineal gland function to some extent, probably partly as a feed-back. The study performed by Radwan *et al*. (49) also proved the role of disturbed MT secretion and metabolism in the pathogenesis of IBS. The authors showed statistically lower 6-HMS/creatinine level in both C-IBS and D-IBS groups compared to the controls.

It is important to note an association between 6-HMS urinary excretion and the symptoms intensity in IBS. It indicates the significant role of MT deficiency in the complex pathogenesis of IBS and justifies usefulness of its therapeutic administration in this disease.

CONCLUSIONS

1. Twenty four-hour 6-HMS urinary excretion in women with the constipation-predominant (IBS-C) as well as the diarrhoea-predominant IBS (IBS-D) is higher than in healthy individuals both in the premenopausal and postmenopausal period.
2. Relatively high 6-HMS urinary excretion in postmenopausal women with IBS-D indicates an adaptive increase in MT secretion from EC cell in the gut.

Conflict of interests: None declared.

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Received: November 16, 2009
Accepted: May 25, 2010

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