

Potential Benefit of Melatonin as an Adjuvant Therapy in a Sample of Iraqi Patients with Crohn's Disease: A New Study

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Abstract:

Inflammatory bowel disease (IBD) is considered as a general term for groups of idiopathic, chronic, relapsing inflammatory disorders of the GIT. Inflammatory bowel disease (IBD) can be divided into two main diseases: Ulcerative colitis (UC) and Crohn's disease (CD). Melatonin (N-acetyl-5-methoxytryptamine) is a hormone that is secreted from a pineal gland and other tissues such as gastrointestinal tract (GIT). This study was intended to evaluate the potential effectiveness of melatonin as an adjuvant to the standard treatment. This interventional prospective randomized-controlled, open-label, single center study was carried out on 30 patients visiting the Biological Unit/Baghdad Teaching Hospital/Medical City Directorate, for their scheduled standard treatment for moderate to severe CD during the period from August 2016 to March 2017. Ethical committee approval and patients written consent were obtained. Hemoglobin (Hb) level in CD patients was significantly increased after both regimen (standard treatment and melatonin adjuvant therapy), but no significant difference in hemoglobin between the two groups. The erythrocyte sedimentation rate (ESR) level was significantly decreased after both regimens ($P < 0.05$) with no significant difference was found between patients groups. The tumor necrosis factor (TNF- α) level was not affected after both regimens in CD patients. From the present study, a promising therapeutic strategy emerged in respect to melatonin as adjuvant therapy in patients with CD which may suggest a role in ameliorating disease process both subjectively and objectively thus optimize therapeutic outcome.

Keyword: Inflammatory bowel disease, Crohn's disease, Melatonin.

الفائدة المحتملة من الميلاتونين كعلاج مساعد في عينة من المرضى العراقيين الذين يعانون من مرض كرون: دراسة جديدة

الخلاصة:

تعتبر أمراض الأمعاء الالتهابية تصنيفاً عاماً لمجموعة من الاضطرابات الالتهابية المزمنة، مجهولة السبب. أمراض الأمعاء الالتهابية يمكن تقسيمها إلى اثنين من الاضطرابات الرئيسية: التهاب القولون التقرحي ومرض كرون. الميلاتونين (N) أسيتيل-5-ميثوكسي تريبتامين (هو هرمون يفرز من الغدة الصنوبرية والأنسجة الأخرى مثل الجهاز الهضمي. تم تصميم هذه الدراسة لتقييم التأثير المحتمل للميلاتونين كعلاج مساعد في المرضى الذين يعانون من مرض كرون المتوسط والشديد وتتبع التغيرات الحاصلة على الالتهابات و الهيموغلوبين. دراسة تداخلية احتمالية عشوائية لتقييم فعالية الميلاتونين في المرضى المشخصين مرض كرون المتوسط والشديد في مركز واحد على 30 مريضاً يزورون وحدة العلاج البايولوجي / مستشفى بغداد التعليمي / مدينة الطب، لاختذ للعلاج المقرر لهم خلال الفترة من أغسطس 2016 إلى مارس 2017. وتم الحصول على موافقة اللجنة الأخلاقية والموافقة الخطية من المرضى. ارتفع مستوى الهيموغلوبين في المرضى كرون بشكل ملحوظ بعد كل من نظام (العلاج القياسي و علاج الميلاتونين المساعد)، ولكن لا يوجد فرق كبير بين المجموعتين. انخفض مستوى ESR بشكل ملحوظ بعد كلا العلاجين ($P < 0.05$) مع عدم وجود فرق ملحوظ بين مجموعات المرضى. لم يتأثر مستوى TNF بعد كلا العلاجين في مرضى كرون. من هذه الدراسة، ظهرت استراتيجية علاجية واعدة فيما يتعلق الميلاتونين كعلاج مساعد في المرضى الذين يعانون من مرض كرون التي يمكن أن يكون له دور ملحوظ في تحسين عملية علاج المرض.

الكلمات الدالة: امراض الامعاء الالتهابية، مرض كرون، الميلاتونين

Introduction

Inflammatory bowel disease (IBD) is a general term for a group of idiopathic, chronic, and relapsing inflammatory disorders of the gastrointestinal tract (GIT) [1].

The IBD prevalence is most noticed in the western countries, influencing up to (0.5%) of the overall population^[2]. Europe was found to have a higher prevalence of ulcerative colitis than crohn's disease, while the reverse had been seen in Australia; Crohn's disease and ulcerative colitis are uniformly distributed in the North America^[2].

The exact cause of IBD is not well identified. Dysregulation of the inflammatory response within the gastrointestinal tract (GIT), genetic predisposition, and environmental or antigenic factors are believed to be involved^[3].

The style of inflammation in Crohn's disease is discontinuous; areas of inflammation are separated with areas of normal GIT mucosa, resulting in characteristic "skip lesions"^[4, 5]. It is present by transmural inflammation, which leads to many problems such as abscesses, fistulas, and strictures ^[6, 7].

In Crohn's disease (CD), a high number of CD4+ T cells found in the intestinal lamina propria of CD yield a lower quantity of interleukin-4 (IL-4) and massive level of interferon- γ (IFN- γ) in comparison to that of healthy control ^[8]. Tumor necrosis factor- α is a main contributor to the inflammatory process in CD, and its physiologic effects involve activation of macrophages, procoagulant properties in the vascular endothelium, and increased production of matrix metalloproteinases in mucosal cells ^[6].

Melatonin(N-acetyl-5-methoxy tryptamine) is a hormone that is secreted from a pineal gland and other tissues such as gastrointestinal tract (GIT). It was discovered in 1958 in the pineal tissue of Bovine ^[9, 10]. The light and dark that

regulate the release of melatonin from pineal gland were received in the suprachiasmatic nuclei by photosensitive ganglionic retinal cells ^[11].

The most predominantly observed effects of melatonin were on the reduction of cell adhesion molecule (CAM), nuclear factor κ B (NF κ B) stimulation, inducible nitric oxide (iNOS) suppressing, and suppression of macrophages. All are causing positive modulation of TNF- α , IL-1 β , IL-6, IL-8, malondialdehyde (MDA), nitric oxide NO, myeloperoxidase MPO, leukocyte infiltration, and COX-2 pathway ^[12].

Melatonin has the ability to protect GIT mucosa against injury by enhancing the immune system, enhance micro-circulation, and epithelial regeneration^[13].

The participation of melatonin in oxygen free radical scavenging was established in different experimental colitis models. Thus melatonin may clarify its protective role in colonic inflammation by the decrease in bacterial translocation which might be linked with the reduced cell apoptosis ^[14].

This study was intended to assess the potential effect of melatonin as an adjuvant treatment in patients with a moderate-severe CD with the current immunosuppressant and biological targeting therapy to reduce the inflammatory marker (erythrocyte sedimentation rate (ESR) and tumor necrosis factor- α (TNF- α)) and improve the hemoglobin (Hb) level.

Patiens And Methods

This is a prospective randomized controlled open-label interventional study to evaluate the potential benefit of melatonin as adjuvant therapy in CD patients. Thirty candidate patients with moderate to severe CD were registered in the study during their visit to Baghdad teaching hospital. The patients were under the supervision of gastroenterologist specialist and were treated according to clinical practice guidelines and disease severity ^[15,16]. Institutional ethical approval was achieved, and patients were issued

with written consent obtained before preceding the study.

The eligible patients were allocated into four main groups:

Group1: Include 15 patients with moderate to severe Crohn's disease (CD) assigned to receive the standard treatment (Infliximab 5mg/kg, azathioprine 50 mg twice daily) for 8 weeks' regimen.

Group2: Include 15 patients with moderate to severe Crohn's disease (CD) assigned to receive standard treatment with melatonin (5mg Melatonin, Infliximab 5mg/kg, azathioprine 50 mg twice daily) for 8 weeks' regimen.

Ten ml of venous blood were collected using a plastic disposable syringe. The blood was kept in a plain disposable tube (gel and clot activator) and was allowed to clot and then separated by centrifuge at a speed of 3000 rpm for 10 minutes. Three ml was collected in k3 EDTA tube and used in complete blood picture test and erythrocyte sedimentation rate (ESR) test. The serum samples were stored in Eppendorf tube at (-80OC) until the time of examination for the other tests. Hemoglobin level was estimated using automated assay by ADVIA 120 Hematology System. The analysis was performed by placing (175 μ L) of the blood sample in the Hematology System. Erythrocyte Sedimentation Rate (ESR) was estimated using automated assay by using the Mixrate-X20 instrument. The analysis was performed by placing 1ml of the blood sample in the ESR autoanalyzer. The results are complete within (30) minutes, correlated to one (1) hour following the Westergren reference method. The reference range is (\leq 15 mm/hr) for men and (\leq 20 mm/hr) for women(17). The TNF- α concentration is determined by using ELISA test. The principle of this test was based on the sandwich type. The

absorbance was estimated at a wavelength of 450 nm. Then TNF- α concentration in the samples was detected proportionally and is determined by using the standard curve(18). The SPSS 24.0 was used to make the statistical analysis. P-values $>$ 0.05 are not significant while P $<$ 0.05 significant and P $<$ 0.01 are highly significant.

Results

Patients demographic and disease characteristics

The present study included 30 patients (10 females (33.3%) and 20 males (66.7%)) with no statistically significant difference found between the study groups concerning both genders P $>$ 0.05, table (1). The age range for all patients were between 18-53 year with the average age of the study groups were 33.47 ± 10.42 years for group 1 patients and 35.27 ± 7.72 years for group 2 patients. No statistical significant difference found between study groups with respect to age P $>$ 0.05. The average body mass index (BMI) for group 1 patients and group 2 patients were 23.31 ± 5.51 kg/m² and 24.61 ± 3.61 kg/m² respectively. No significant difference was found between the study groups with respect to the BMI P $>$ 0.05. The duration of the disease for patients in group 1 and 2 were as follows: 60% versus 53.3% for less than or equal 2 years duration, 13.3% versus 33.3% for 2-4 years duration, 13.3% versus 0% for 4-6 years duration, 13.3 versus 13.3 for more than or equal 7 years. The mean was 3 ± 2.48 years and 3.07 ± 2.15 years for group 1 patients and group 2 patients respectively. No significant difference was found between both groups with respect to the duration of the disease P $>$ 0.05.

Table -1: Patients demographic and disease characteristics

Study groups			
Variable	Group 1	Group 2	P-value
Gender	n (%)	n (%)	-
Female	6 (40)	4 (26.7)	0.439 ^{NS}
Male	9 (60)	11(73.3)	
Total	15 (100)	15(100)	
Age(year)	33.47 ± 10.42	35.27 ± 7.72	0.595 ^{NS}
Length(cm)	166.73 ± 12.22	173.93 ± 9.75	0.085 ^{NS}
Weight(kg)	64.67 ± 15.06	74.33 ± 11.71	0.060 ^{NS}
BMI(kg/m²)	23.31 ± 5.51	24.61 ± 3.61	0.451 ^{NS}
Duration of disease(year)	n (%)	n (%)	0.938 ^{NS}
≤ 2	9(60)	8(53.3)	
2-4	2(13.3)	5(33.3)	
4-6	2(13.3)	0(0)	
≥7	2(13.3)	2(13.3)	

Data presented as mean ± SD

Number of patients (n), Percentage (%) NS: No significant differences ($P>0.05$)

Effect of melatonin adjuvant therapy and standard treatment on Hb level in CD patients

There was no significant difference in the mean HGB between group 1 and group 2 patients at baseline and after 8 weeks of study $P>0.05$. In both study groups 1 and

2a significant increase in mean HGB level was seen after 8 weeks compared to pre-treatment level $P<0.05$. Overall, there was no significant difference between the study groups after the end of the study period (P -value of interaction =0.855), table-2.

Table-2 Effect of melatonin adjuvant therapy and standard treatment on Hb level in CD patients

Variable	Study groups		
	Group 1	Group 2	P-value
Hb (g/dL)			
Pre-treatment	12.96 ± 2.42	12.89 ± 2.12	0.942 ^{NS}
Post-treatment	13.71 ± 2.41	13.73 ± 1.88	0.984 ^{NS}
P-value	0.010*	0.026*	0.855 ^{aNS}
Percent change	5.8%	6.5%	-

Data presented as mean ± SD.

Number of patients (n), Percentage (%) (*) Significant difference ($P<0.05$)

a: is P -value of interaction

Effect of melatonin adjuvant therapy and standard treatment on ESR and TNF- α in CD patients

There was no significant difference in the mean ESR between group 1 and group 2 patients at baseline and also after 8 weeks of treatment $P>0.05$. Significant decrease in mean ESR in group 1 patients $P<0.05$

and a highly significant decrease in group 2 patients $P<0.01$ were seen after 8 weeks of treatment compared to pre-treatment level in each group, with up ceiling percentage of change in group 2 patients on melatonin supplement -50.3% compared to group 1 patients -28.4%. Still, there was no significant difference between the groups after end of study

period (P -value of interaction =0.241), table (3).

There was no significant difference in the median TNF- α between group 1 and group 2 patients both at baseline and after 8 weeks of treatment $P>0.05$. No significant increase in TNF- α was seen after 8weeks

compared to pre-treatment level in both study groups as well $P>0.05$. The overall result revealed that there was no significant difference between the patient groups in respect to TNF- α level at the end of study period (P -value of interaction =0.952), table-3.

Table-3 Effect of melatonin adjuvant therapy and standard treatment on ESR and TNF- α in CD patients

Variable	Study groups		
	Group 1	Group 2	P-value
ESR (mm/hr)			
Pre-treatment	24.87 \pm 20.84	24.27 \pm 16.22	0.931 ^{NS}
Post-treatment	17.80 \pm 13.97	12.07 \pm 6.17	0.162 ^{NS}
P-value	0.027*	0.002**	0.241 ^{aNS}
Percentage change	-28.4%	-50.3%	-
TNF-α (pg/ml)			
Pre-treatment	2 (1.8 – 3.1)	2.4 (2.2 – 3.3)	0.250 ^{NS}
Post-treatment	2.4 (1.8 – 3.4)	2.9 (2.1 – 3.8)	0.345 ^{NS}
P-value	0.712 ^{NS}	0.460 ^{NS}	0.952 ^{aNS}
Percentage change	9.8%	10.7%	-

Data presented as mean \pm SD and median (IQR).

a: is P -value of interaction

NS: No significant differences ($P>0.05$), (*) Significant difference ($P<0.05$), (**) Highly Significant difference ($P<0.01$).

Discussion

In the present study, all Inflammatory bowel disease (IBD) patients were aged between 18-53 years, and both genders were enrolled in the study with a slight predominance of male over female in the study (67%). This was consistent with the previous study in Japan where male-to-female percent in CD was nearly (70%)^[19]. Several studies from Western countries stated that the prevalence of adult CD is equal or higher with women adult females^[7, 20, 21].

One of the routine investigation for IBD patients was hemoglobin level which was recommended that even patients in clinical remission should be tested every 12 months, whereas patients with active IBD should be tested for anemia at least every 3 months^[22].

Crohn's disease patients in this current study were presented with near normal levels of Hb before treatment. This level was notably increased after treatment with both standard therapy and melatonin

supplementation and percent of change in hemoglobin level that nearly equal in both groups, suggesting the minimal impact of melatonin on Hb homeostasis.

Bergamaschi *et al.* have observed that standard treatment with infliximab had an effect on improving anemia through the regulation of disease activity and inflammation, in which infliximab has increased erythropoietin production to more adequate for the hemoglobin level, therefore allowing more efficient bone marrow activation^[23]. Also, they found that interleukin-6, but not the TNF- α , is a promoter of hepcidin formation (a key controller of the entry of iron into the circulation in mammals which was pointed to have anti-inflammatory properties)^[24], leading to irregular iron homeostasis in the inflammation; may infliximab directly modifies IL-6 or through hepcidin formation, the net effect is improving erythropoiesis in IBD patients^[23].

In 2011 Chojnacki *et al.* found in a clinical study on patients with UC that there a

substantial decrease in Hb levels in blood from (12.05±0.69) to reach (10.93±0.81) g/dl after receiving placebo. Meanwhile, melatonin adjuvant produced a slight reduction in Hb levels in blood from (12.29±0.87) to (11.76±1.09) g/dl^[25].

Melatonin was approved to have a high antioxidant activity, anti-inflammatory activity, and high melatonin concentration in the GIT would be anticipated to decrease and/or improve tissue damage^[12]. The protective mechanisms of melatonin have not been fully clarified. Melatonin seems to participate in many defense mechanisms in colonic inflammatory cascade because; it stimulates the immune system, improves the gut blood flow, preserves the important endogenous antioxidant store of GSH, prevents lysosomal enzyme disturbance, reduces the levels of TNF- α , decreases the free radical levels, inhibits the enhanced myeloperoxidase activity, induction of the apoptotic processes and decreases the bacterial translocation levels, thus reducing the extent of colonic damage^[26].

Crohn's disease patients in the present study have shown a notable decrease in ESR after conventional therapy alone ($P<0.05$) and marked decrease after melatonin adjuvant therapy ($P<0.01$), although did not reach the statistical significance between the groups. Patients on melatonin adjuvant therapy, the ESR has reached the reference range (≤ 20 mm/hr) for women and (≤ 15 mm/hr) for men^[17]. These findings suggest a potential effect of melatonin compared to standard therapy, although no data are available to explain this result.

The pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) seems to be central factor in the inflammatory progression of IBD⁽²⁷⁾. Activated macrophages mostly produce TNF- α , but also is released by monocytes and T cells⁽²⁸⁾. There was an elevated TNF production in the GIT mucosa of patients with CD, and TNF level are raised in the feces of active IBD patients^[27]. The

influence of TNF- α as an initiator of inflammation comes from its stimulation of the "master switch" inflammatory transcription mediator, nuclear-factor- κ B (NF- κ B)^[29]. Direct effects of NF- κ B stimulation involve increases in GIT permeability, increased production of other pro-inflammatory cytokines and mediators, upregulation of cell adhesion molecules(CAM), and cells apoptosis⁽²⁸⁾.

In the present study, CD patients revealed an increased TNF in both groups following standard therapy alone and on melatonin adjuvant therapy with percentage of increment (9.8% vs. 10.7%) respectively.

The change in the level of TNF considered not significant among all groups ($P>0.05$). Moreover, at baseline and 8 weeks of treatment, the TNF level in all study patients was below and within the reference range which is <5.6 pg/ml^[30]. So even that melatonin had the effect to decrease or increase TNF level, it would be difficult to detect the clear effect because of the powerful effect of standard therapy specially infliximab that maintained TNF level within normal range. Probably using melatonin as a mono interventional therapy for longer time or higher dose, which was one of the limitations of the study, may be required to verify the clear effect on TNF.

The vast majority of studies exploring the potential mechanism of melatonin in the inflammatory process with respect to IBD were experimental models or animal studies. In the previous experimental studies by Mei *et al.* who reported that melatonin decreased colon mucosa damage and fecal occult blood similar effect to that of 5-ASA, decreased the levels of IL-1, TNF, and NO^[31]. Also, Li *et al.* found that melatonin had a highly significant effect on reducing the level of TNF in a rat colitis model which was induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS)^[32]. Mazzon *et al.* revealed a marked suppression of TNF- α and IL-1 β levels in the colon mucosa from DNBS-induced colitis in rats treated with melatonin^[33]. In 2015, Park *et al.* stated that melatonin had

significantly reduced the level of IL-1 β , IL-6, IL-17, TNF- α , and INF- γ in the plasma of the experimental mice with colitis which induced with DSS with sleep deprivation^[34].

Conclusion

From the present study, a promising therapeutic strategy emerged in respect to melatonin as adjuvant therapy in patients with CD which may suggest a role in ameliorating disease process both subjectively and objectively thus optimize therapeutic outcome.

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