Serum Interleukin-23 Levels in Patients with Ulcerative Colitis

Mojgan Mohammadi[1*], Mohammad Mahdi Hayatbakhsh[2], Mohammad Javad Zahedi[2], Mahmood Reza Jalalpour[2], Amin Pakgohar[3]

[1]Physiology Research Centre, [2]Gastroentrology Department, Afzalipour Hospital, Kerman University of Medical Sciences, [3]Kerman Blood Transfusion Centre, Kerman, Iran

ABSTRACT

Background: Patients with ulcerative colitis are at increased risk of inflammation. Interleukin 23 (IL-23) is a newly identified cytokine with increased expression in inflamed biopsies of colon mucosa in patients with Crohn's disease; however, there is inconsistent evidence on its role in ulcerative colitis. **Objective:** We aimed to compare serum IL-23 level in patients with ulcerative colitis and normal controls and determine if serum IL-23 level increases with the severity of disease according to endoscopic findings. **Methods:** We quantified serum IL-23 levels from 60 patients with ulcerative colitis and 20 control individuals. All patients underwent endoscopic procedure to define the severity of disease. Patients were then stratified into 2 groups of "Mild" and "Severe" according to the endoscopic findings. **Results:** For comparison of serum IL-23 levels, Platelet count, ESR and CRP between the groups, Mann-Whitney U test and independent sample t test were employed, as appropriate. Pearson's and spearman's correlation tests were employed to test the association of IL-23 with platelet count, CRP and ESR in patients. Our findings showed that serum IL-23 levels were increased in patients with ulcerative colitis compared to normal control. Moreover, patients in "Severe" group had higher serum IL-23 levels and ESR compared with those in "Mild" group. There was no significant sexual dimorphism in any of studied variables. **Conclusion:** We suggest that IL-23 plays an important role in the pathogenesis of ulcerative colitis and is a marker of disease activity in these patients.

Keywords: Inflammation, Interleukin-23, Ulcerative Colitis

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*Corresponding author: Dr. Mojgan Mohammadi, Physiology Research Centre, Kerman University of Medical Sciences, Kerman, Iran, Tel: (+) 98 3412114098, Fax: (+) 98 3412114760, e-mail: mozhganmohammadi69@yahoo.co.uk

and dendritic cells in response to pathogenic antigenic challenge and is essential in driving early local immune response (2,3). It has a unique role in the maturation of helper T cells and differentiation of memory T cells (1,2). Additionally it regulates pro-inflammatory cytokines, which are important in cell mediated immunity against intracellular pathogens (2,4). IL-23-driven inflammation has primarily been linked to the actions of a new subset of T-helper cells namely T helper type 17 (4,5). They not only play an important role in host defense against extracellular pathogens, but are also associated with the development of autoimmunity and inflammatory response such as inflammatory bowel disease (IBD) (5).

It is known that both ulcerative colitis (UC) and crohn disease (CD) are severe intestinal inflammatory conditions which both are resulted from autoimmune and immune mediated phenomena (6). It is suggested that CD is a predominantly T helper 1 and T helper 17 mediated process, while UC is mainly mediated through T helper 2 and natural killer cells (6). IL-23 promotes the development T helper 17 cells from mechanisms that are distinct from those of T helper 1 (6). It is usually considered as a cytokine mediating CD disease (6,7); however recent studies have found increased expression of IL-23 in inflamated and noninflamted mucosa of patients with UC (8). Similarly genome wide associated studies have linked UC to a number of IL-23 pathway genes (9). However the role of IL-23 in the pathogenesis of UC in not elucidated yet.

Studies have repeatedly shown UC as an autoimmune disorder (10). IL-23 gene polymorphism is also identified as a susceptible gene for the development of multiple autoimmune disease (11-13). On the other hand studies have suggested that increase in markers of oxidative stress is associated with the activity and severity of UC (14,15). IL-23, a proinflamatory cytokine proposed to be central in the development of autoimmune disease and is shown to be essential in the initiation of autoimmune tissue inflammation (16-18). So it could be hypothesized that UC is associated with increased levels of IL-23 both resulted from increased inflammation and autoimmunity. Here we aimed to 1: Compare serum IL-23 level in patients with ulcerative colitis and normal controls and 2: defining the correlation between serum IL-23 levels and disease activity in patients with UC.

MATERIALS AND METHODS

We performed a cross sectional analysis of 60 patients with UC from gastroenterology clinic of Afzali Pour hospital affiliated to Kerman university of Medical Science plus controls. UC was diagnosed according to the criteria of American Gastroenterology Association (19). All patients underwent endoscopic study to define the severity of disease. Patients were then stratified into 2 groups of "Mild" and "Severe" according to endoscopic findings. Serum samples were collected from all participants. Exclusion criteria were pregnancy, acute or chronic renal failure, congestive heart failure, thyroid disorders, acute infections, and stroke and hospital admission in recent months. Demographic data including age, sex, duration of disease, history of cigarette smoking, history of drug abuse and family history of disease was also taken from the patients.

Blood Samples. Blood samples were collected after 12 hours of fasting and, serum Palettes, Erythrocyte Sedimentation Rate (ESR) and C Reactive Protein (CRP) were

measured. Platelets measurements were carried out using sysmex automated cell counter (Kx21n, Japan). ESR was determined using ESR-reader automated (Linear, Spain). CRP was measured Latex agglutination method (Bionik, European Union). Serum IL-23 was measured using ELISA kit (Bendermed, 2009, Austria). Intra and interassay coeeficeitn respectively 5.9% and 8.2%.

Statistical Analysis. The statistical package SPSS 16 for windows (Chicago, Illinois, USA), was used for analysis. Kolmogorov-Smirnov test was employed to test the normality of the variables in each group. Variables distributed normally are presented as mean ± standard error of mean (SEM). Variables with skewed distribution are presented as median (interquartile range). For comparison of serum IL-23 levels, Platelet count, ESR and CRP between groups of patients and controls, Mann-Whitney U test and independent sample T test were employed, as appropriate. Pearson's and spearman's correlation test were employed to test the association of IL-23 with platelet count, CRP and ESR in patients.

Ethical Considerations. The study protocol was approved in ethics committee of Kerman University of Medical Sciences and each patient gave informed consent before enrollment in the study.

Table 1. Primary characteristics of participants.

	Control (n=20)	Patients in "Mild" group (n=23)	Patients in "Severe" group (n=37)
Female (n, %)	9 (45.5%)	16 (76.2%)	18 (48.6%)
Disease Duration (yrs)	-	2.90 ± 2.508	2.78 ± 4.077
Age (yrs)	35.4 ± 13.9	34.00 ± 13.921	39.46 ± 17.066
Erythrocyte Sedimentation Rate (mm/hr)	10.32 ± 5.7††	19.52 ± 14.736	28.11 ± 13.800**
Platelets	266.430 ± 6.89	227.33 ± 77.051	267.54 ± 139.759
Hemoglobin (mg/dl)	12.9 ± 2.31	12.729 ± 2.3326	12.173 ± 2.8962
Interleukin 23 (pg/ml)	280 [193 - 237]†	300 [254 - 311]	320 [318 - 417]*

†p<0.05 when comparing control with patients , * p<0.05, **p<0.01 when comparing patients with mild involvement of mucosa with those of intermediate to severe involvement .

RESULTS

Characteristics of participants are presented in table 1. Our findings showed that serum IL-23 levels were increased in patients with ulcerative colitis compared to normal control. We also demonstrated that patients in "Severe" group have higher serum IL-23

level and ESR compared with those in "Mild" group (figure 1). There was not any significant sexual dimorphism in any of studied variables. There was also a significant negative correlation between serum IL-23 and age in patients with ulcerative colitis (r=-0.33, p<0.05)

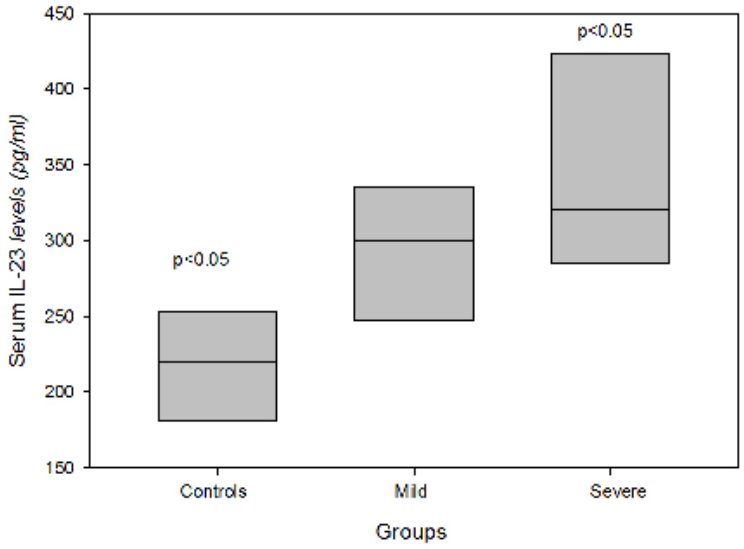

Figure 1. Serum interleukin 23 levels in 2 groups of patients: "Mild" and "Sever" according to endoscopic findings of UC severity, plus controls. For comparison of serum IL-23 levels between groups of patients and controls, Mann-Whitney U test and independent sample T test were employed, as appropriate.

DISSCUSION

Our findings demonstrated that serum IL-23 was increased in patients with UC compared with normal controls. We also showed that serum IL-23 were associated with disease activity in UC patients. Serum IL-23 was also negatively correlated with age in patients with ulcerative colitis. Although these findings does not allow us to determine whether IL-23 is a link in the pathogenesis or severity of UC, it supports the hypothesis that increased IL-23 level reflect the activity of T helper 17 in patients with UC.

These findings confirm those from previous studies. In consistent with our findings it is shown that serum IL-23 participates in the pathogenesis of ulcerative colitis (20). The authors suggested that IL-23 may play a critical role in the development of UC through inducing production of IL-17 (20). Likewise it is shown that an increased expression of IL-23 in intraepithelial tissue promotes inflammation. Hence it is suggested that targeted therapy against IL-23 may have therapeutic effects in patients with UC (21-23). Kobayashi et al. showed that IL-23 receptor is upregulated in CD4+ T cells from both UC and CD (8). They also showed the significant role of T helper 17 in UC by the finding that recombinant IL-23 actually enhance IL17 production by CD4+ T cells in lamina propria, when it had a lesser effect in CD (8), Schmidt et al. showed up

regulation of IL-23p19mRNA in colonic mucosa of CD and to a lesser extent in UC (20). These findings support our findings that increased IL-23 plays an important role in the pathogenesis of UC. However Fuss et al. showed that macrophages from CD patients, but not UC patients, produce large amounts of IL-23 (21).

Many Studies have shown IL-23 as an inflammatory marker. It is significantly increased in many autoimmune disease including , rheumatoid arthritis, psoriasis, systemic lupus erythematous, multiple sclerosis and goodpasture (22-26). So we hypothesized that increased IL-23 level may be a marker of disease activity in patients with UC. Interestingly we found a significant difference in serum IL-23 level of groups stratified according to disease activity. Similarly Kader at al reported that specific circulatory cytokines including IL-12 and TGF-beta1are increased in remission stage of pediatric inflammatory bowel disease compared with those in active disease (27). Besides there was not any significant correlation between markers of inflammation including C reactive protein, and erythrocyte sedimentation rate with serum IL-23 level. This could be due to the fact that none of these biomarkers biosynthesizes via similar pathways or simultaneously owing to their diverse nature and origin in the pathogenesis UC.

We also found a significant negative correlation between serum IL-23 and age of the patients. Previous studies have reported decrease in cell mediated immunity including T and B cell function in aging. We did not found any study demonstrating the direct role of aging on serum IL-23 levels. However El Mezayen et al. reported increased expression of p19 subunit of IL-23 during aging (28). However they did not measured serum IL-23 level of their studied population, furthermore our studied populations were suffering from ulcerative colitis when this report is from normal people.

In conclusion, we have shown increased serum IL-23 levels in patients with UC. Moreover increased serum IL-23 levels show the disease activity in these patients. weather these findings are due to increased inflammation or an important role of T helper17 in the pathogenesis of UC, have to be studied in future.

The Principal limitation of the present study is its cross sectional nature which prelude the determination of the direction of causality, however we took advantage of a relatively large sample size and close similarity between groups in most of the confounding variables.

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