Original Article
Serum Interleukin-33 level in Saudi children with inflammatory bowel disease

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Abstract: Interleukin-33 (IL-33) is a cytokine that belongs to the interleukin-1 family and has been shown to be associated with mucosal inflammation. The aim of this study was to determine the serum level of IL-33 in children with ulcerative colitis (UC) and Crohn’s disease (CD) and to correlate the level with the disease progression. In this cross sectional prospective study, we enrolled 50 children with IBD from KAUH, Jeddah, Saudi Arabia and 34 healthy control subjects between June 2012 and December 2012. Serum IL-33 was assessed by ELISA and CRP by immunonephelometric assay. Results from our study showed 32 CD and 18 UC patients included. The median age was 13.5 years for CD patients, 11.9 years for UC patients and 11.2 years for controls. Females constituted 53%, 66.7% and 59% of CD, UC and control subjects respectively. The median serum IL-33 in UC patients of 55.5 pg/mL was significantly higher than the median IL-33 level of 41 pg/mL in the healthy control (P=0.04) but no significant difference was found between the median IL-33 level in the sera of CD and the control group (P=0.7). A higher median IL-33 level was also found in active disease (P=0.03). In our cohort, the serum level of IL-33 was positively correlated with hs-CRP (r=0.48, P < 0.001). To conclude, our results support that serum IL-33 level is increased in children with UC as compared with control. Serum level is correlated with the disease activity; therefore it could be used as a potential biomarker for monitoring the severity of the disease in children with UC.

Keywords: IL-33, ulcerative colitis (UC), Crohn’s disease (CD), children, Saudi Arabia

Introduction
Inflammatory Bowel Disease (IBD) is a group of chronic relapsing remitting inflammatory conditions of the gastrointestinal tract. It comprises of ulcerative colitis (UC) that affect solely the mucosa of the colon and rectum and Crohn’s disease (CD) that may involve any part of the gastrointestinal tract, and is associated with discontinuous transmural lesions of the gut wall. Both are characterized by overall epithelial barrier dysfunction and unrelenting leakiness consequential from dysregulated immune responses. Several recent studies have predicted significant increase in IL-33 expression in the inflamed mucosa of IBD patients in comparison to healthy controls, more predominantly in UC.

The pathogenesis of IBD was open after the discovery of Interleukin-33 (IL-33) in 2005, recognized with abundant and pragmatic immunomodulating effect of this cytokine in diversity of cells. In the current era, Interleukin-33 (IL-33) is an illustrated member of the IL-1-family of cytokines, and it is a ligand of the ST2 receptor. Schmitz et al. established that NF-HEV is a member of the IL-1 cytokine superfamily and shared several molecular properties with IL-1a/b (IL-1F1/IL-1F2), IL-1Ra (IL-1F3), and IL-18 (IL-1F4). At this time, we have little comprehension about its authentic role except that it may perhaps help in controlling the specialized phenotype of HEV. However, its presence is also established in antigen-presenting cells such as macrophages and dendritic cells. Moreover, IL-33 mRNA expression levels have been shown in different tissue and organs including spleens and the central nervous system. It has been suggested that IL-33 is released through cell necrosis; it is bioactive and triggers inflamma-
tion in an autocrine or paracrine manner. It could be a novel target for the treatment of a variety of diseases; many earlier studies have demonstrated that IL-33 perhaps encompasses a pleiotropic role in diverse diseases. Recent works have demonstrated the role of IL-33 in chronic autoimmune and cardiovascular diseases.

Consequently, the effects of IL-33 could be either pro-inflammatory or anti-inflammatory depending on the severity and condition of disease. The present studies are suggestive of IL-33 as distinctively unrestrained during cellular death due to necrosis and considered to be coupled with tissue damage during infection or trauma. Considering these properties, IL-33 has been predicted to alert the immune system of any endogenous danger after infection or trauma or in intracrine way it acts as a negative regulator of NFκB gene transcription. This led to numerous studies exploring its immunomodulatory function. In addition to this, involvement of IL-33 was shown in the modulation of inflammation, since it can prop up inflammatory and fibrotic disorders of gastrointestinal tract, rheumatic and airway inflammatory diseases and anaphylactic shock. Signaling in the course of ST2 emerge to be triggered through the cytoplasmic Toll-interleukin-1 receptor (TIR) domain of IL-1 accessory protein (IL-1 RAcP). This eventually leads to recruitment of the adaptor protein MyD88 and activation of NF-kB through TRAF6, IRAK1/4 and MAP kinases. Therefore, IL-33 appears to be a cytokine with dual role, through activation of the ST2L receptor complex as well as an intracellular nuclear factor with transcriptional regulatory properties.

Hence, the aim of this study was to assess the expression of serum IL-33 in children with UC and CD and to correlate the serum level with the disease activity.

Materials and methods

**Patients and controls**

In this cross sectional prospective study, we enrolled 50 children and adolescents with clinical diagnosis of IBD seen at the Paediatric Gastroenterology Clinic at King Abdulaziz University Hospital, Jeddah, and 34 healthy control subjects during the period from June 2012 through December 2012. A written informed consent was obtained from the children's parents or guardians before enrollment. The diagnosis of UC and CD was based on the international criteria established by the working groups of the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and North American Society for Pediatric Gastroenterology Hepatology and Nutrition (NASPGHAN) depending on a combination of clinical, laboratory, imaging, endoscopic, and histopathology features. The extent of the disease in UC patients was classified based on Montreal classification into distal colitis (mucosal changes limited to the rectum and sigmoid), left sided colitis (mucosal changes beyond the splenic flexure), or extensive colitis (mucosal changes beyond the splenic flexure). The disease phenotype in CD was classified according to Vienna classification into small
bowel disease (L1), isolated colonic disease (L2) and ileocolonic disease (L3). For UC patients Mayo UC endoscopic score was used for assessment of mucosal inflammation.

Clinical disease activity was assessed using the abbreviated Pediatric Crohn’s Activity Index (abbrPCDAI) for patients with CD. A cut-off score of < 10 in the abbrPCDAI define remission. For patients with UC, Pediatric Ulcerative Colitis Activity Index (PUCAI) was used to assess the disease activity. A score of < 10 denotes disease remission.

Clinical data for IBD patients were obtained retrospectively from their medical records. The control group comprised of healthy children and adolescents who had no history of chronic illness including immune mediated disorders. The study was approved by the Bioethical and Research Committee at Faculty of Medicine at King Abdulaziz University, and the study was conducted according to the principles of Helsinki Declaration.

Preparation of patient’s samples

Blood samples were collected from patients and controls in EDTA tube that was stored in -80 until processed. Sera were separated by centrifugation at 2000 rpm for 2 minutes.

Measurement of IL-33 by ELISA

Serum samples were assessed by ELISA. The ELISA method was established according to the commercial kit protocol (DuoSet ELISA development system; R&D; USA). ELISA plate was coated with 100 µl of diluted capture antibody and was sealed and incubated overnight at room temperature. Then the antibody was removed and the plate was washed with washing buffer and then 300 µl of the reagent diluents was added to each well and incubated for 1 hour at room temperature. Then 100 µl of serum samples and standard were added and the plate was covered with adhesive strip and incubated for 2 hours. The plate was washed many times and this was followed by addition of 100 µl of detection antibody to each well and incubated for 2 hours at room temperature. After incubation 100 µl of working dilution conjugate (streptavidin-HRP) was added to each well and incubated for 2 hours. Substrate was added for color development. Finally, stop solution was added for blocking the development of color and the plate was analyzed using the ELISA reader. Calculation of results was done using standard curve which was plotted to determine the regression analysis.

High-sensitivity C-reactive protein (hs-CRP) method

High-sensitivity C-reactive protein (hs-CRP) was measured by an immunonephelometric assay on a BN II analyzer (SIEMENS, Marburg, Germany) using SIEMENS kit for hs CRP assay. This assay is based on particle-enhanced immunonephelometry and it enables the measurement of CRP concentrations as low as 0.175 mg/l. The assay was based on the measurement of polystyrene particles coated with monoclonal antibodies specific to human CRP that aggregate when mixed with samples containing human CRP. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. However, a blood sample was collected and serum was separated and diluted at 1:20 by N diluents’. Siemens control sera was used as recommend-
ed by the manufacturer. Results were calculated using the standard curve that is automatically plotted using a standard sample obtained from the manufacturer insert.

Statistical analysis

All statistical analyses were performed using SPSS 19 software (SPSS, Inc, Chicago, Ill). Data were expressed as the percentage of the total for categorical variables, as the mean with standard deviation (SD) for normally distributed continuous variables. Data with parametric distribution were analyzed by Student’s t test or the Mann-Whitney U test with non parametric distribution. Correlations between each variable were evaluated using Spearman rank-order correlation test. All statistical analyses utilized a 0.05 level of significance.

Results

This study includes 50 patients with IBD (32 CD and 18 UC) and 34 control subjects. The median age was 13.5 years (range 1.3-16.4 years) for CD patients, 11.9 years (range, 5-18.3 years) for UC patients and 11.2 years (2.8-17.1 years) for controls. Females constituted 53%, 66.7% and 59% of CD patients, UC patients and controls respectively. Clinical and demographic characteristics for both CD and UC patients are shown in Tables 1 and 2.

IL-33 levels in relation to clinical disease activity

The median IL-33 levels were compared between IBD group with activity indices less than 10 for either abbrPCDAI or PUCAI and groups with higher activity indices indicating clinically active disease. The median IL-33 level in sera of patients with flare up (n=16) of 110 pg/mL (range, 27.5-493.8 pg/mL) was significantly higher than the median IL-33 level of 39 pg/mL (range, 5.5-72 pg/mL) in patients in remission (n=34), U=168, P=0.03 Figure 2.

Correlation of the IL-33 level with CRP

The correlation of the serum level of IL-33 was also tested against CRP that was considered a marker of monitoring the disease activity in IBD patients. The serum level of IL-33 was positively correlated with the CRP level (r=0.48, P < 0.001) Figure 3.

Effect of treatment with anti-TNF-alpha medications on the serum level of IL-33

On further examination of the effect of biological therapy with anti-TNF-alpha medications on serum IL-33 levels, we compare the IL-33 levels between two groups (a group who was treated with anti-TNF-alpha (n=17) and a group who did not receive treatment with anti-TNF-alpha medications (n=33). We did not find statistically sig-
significant difference between the two groups (median IL-33 level of 45 pg/mL vs. median IL-33 level of 51 pg/mL, U=261.5, P=0.69).

Discussion

In this prospective study of IBD with striking representation of patients of both CD and UC with distinct disease localization and varied disease behavior, suitably judged against healthy controls, we observed significant increase in serum IL-33 in patients of IBD in comparison with control group, nevertheless, this finding was highly pronounced more in favor of UC rather than CD. Recent reports indicates that throughout active UC, distinctive escalation of IL-33 was observed in intestinal epithelial cells as well as in infiltrating lamina propria mononuclear cells, which belongs to the monocyte/and B-cell families. Consistently, amplification of IL-33 serum concentrations were also observed in IBD patients compared to healthy controls of same age cohorts.

Our findings are supported by several studies reminiscent of up regulation of IL-33 mRNA expression in human biopsy specimens from untreated or active UC patients compared with healthy controls have been testified by several studies. Furthermore, it was reported that, in UC the major location for IL-33 expression was found in sub epithelial myofibroblasts (SEMFs) below the ulcerative lesion, but not observed in CD patients, indicative of the potential role for IL-33 in wound/ulcer healing, which may be different in UC compared with CD. Similarly, ST2 transcripts have been reported in mucosa samples from patients with active UC. The intestinal tissue expression of ST2 was significantly different in healthy mucosa compared with chronically inflamed IBD patients, in which ST2 was abundantly expressed in non-inflamed colon epithelium, while during chronic inflammation its expression was lost, reduced or redistributed.

Another key finding in our study was a link between the progression of the disease activity in both UC and CD with the serum levels of IL-33 on the basis of its analysis by the hs-CRP levels. This observation of serum levels of IL-33 in IBD patients has been supported by the results of recent studies which revealed in SAMP1/YitFc (SAMP) mice, an experimental model of DC ileitis, illustrated by an early Th1 immune response and Th2 cytokines dominated late and chronic phase of disease process. It is quite relevant that escalated serum levels of IL-33 in IBD patients replicate an active inflammatory condition and symbolize a prospective biomarker for the disease activity. In addition, we also observed that irrespective of biological therapy, there was no divergence in the serum levels of IL-33 among the IBD group throughout the phase of the assessment. A limitation of our study is that the numbers of patients and controls available for study were relatively small which could decrease the statistically significant relationship from the data.

In conclusion, serum IL-33 expression was increased in children with UC as compared with control. Therefore increased serum IL-33 level could be used for differentiating CD from UC in patients with indeterminate colitis. Serum level was positively correlated with the disease activity; therefore, it could be used as a potential
biodmarkar for monitoring disease progression in children with UC.

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Disclosure of conflict of interest

None.

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