INTRODUCTION
The innate immune system activation is involved in immunological response mechanisms of inflammatory cells against invading pathogens. However, the Inflammasome protein complex composed from the nucleotide-binding domain and leucine-rich repeat (LRR)-containing (NLR) family and the pyrin and HIN domain (PYHIN) family [1]. NLRs are a family of intracellular innate immune recognition molecules [2]. These receptors found to play essential roles through interaction with microbes and environmental stimuli in mucosal immunity and inflammation [3]. NLRP3 is the best characterized member belongs to this family, its fusion with apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), and caspase-1 to form large complex protein named as ‘inflammasome’ resulting in production of inflammatory cytokines IL-1β and IL-18 [4]. Several studies highlighted the pathogenic role of NLRP3 in experimental intestinal inflammation [5-6]. However, NLRP3 deficient mice experienced less sever pathological changes compared to wild type mice, indicating the essential role for IL-1β and IL-18 production could exacerbate DSS mice model [7]. Interestingly, mice deficient model of NLRP3 and ASC displayed higher rate of bacterial colonization, increased tissue pathology and sever weight loss, indicating early activation of NLRP3 and its role in prevention of bacterial colonization on intestinal epithelium as an essential innate immune defense mechanism as well as tissue repair after injury [8].

THE AIM
This study aimed to evaluate the NLRP3 immunoreactivity in ulcerative colitis in various clinical presentations.

MATERIALS AND METHODS
STUDY DESIGN AND SETTING
This case-control study involved 50 ulcerative colitis patients and 30 subjects with negative endoscopic findings. All subjects were recruited from three hospitals in Baghdad: The Gastroenterology and Hepatology Teaching Hospital, Medical city and Al-Emamain Al-Kadhemain medical city as well as private hospitals in the period March 2018- June, 2019. Diagnosis of cases was done according of the guideline of diagnosis and treatment of inflammatory bowel disease [9] in terms of clinical, endoscopic, radiographic and histopathological evaluation. All patients were either newly or previously diagnosed. Demographic data were collected through direct interview with the patient, and by seeking his/her hospital record as well as previous medical reports in addition to the score for UC.

NLRP3 IMMUNOHISTOCHEMISTRY STAINING
All tissue biopsies were processed as formalin fixed paraffin embedded tissue. They were cut into 5 micrometers on positive charge slide (Fisher brand). After removal of wax and sufficient rehydration step, antigen retrieval made by

EVALUATION OF NLRP3 INFLAMMASOME PROTEIN EXPRESSION IN ULCERATIVE COLITIS

Manar Mustafa
MIDDLE TECHNICAL UNIVERSITY, BAGHDAD, IRAQ

ABSTRACT
The aim: This study aimed to evaluate the NLRP3 immunoreactivity in ulcerative colitis in various clinical presentations.

Materials and methods: The retrospective study involved 80 formalin fixed paraffin embedded tissue blocks. These were divided into 50 samples of ulcerative colitis and 30 with normal colonoscopy findings. NLRP3 protein expression patterns were evaluated in various cellular components in tissue sections using immunohistochemistry method.

Results: NLRP3 inflammasome was significantly expressed with higher percentage among ulcerative colitis tissue sections compared with normal tissue sections. Intense staining was observed in Paneth cells at the base of crypts, inflammatory cells infiltrated between glands and near the base of crypts. Variable intensities of NLRP3 staining were observed in surface epithelial cells and glandular epithelium. Statically higher percentage of expression was found among active disease and patients with extra-intestinal complications.

Conclusions: The NLRP3 protein expression pattern was upregulated among various cellular compartments among ulcerative colitis and correlated with disease activity.

KEY WORDS: NLRP3 protein, immunohistochemistry, ulcerative colitis
autoclave for 3 minutes using citrate buffer pH: 6.0. Endogenous peroxidase was blocked, and protein block was added on slide, Cryopyrin antibody (orb182473) purchased from Biorbyte® diluted as 6 pg/ml of antibody diluents (orb90427) and added on slide (50 micoliter), and stained by Super Sensitive IHC Detection System Kit (orb219874) Biorbyte®, Counterstained by Myers hematoxylin (Dako). Both intensity score was classified as 0=negative, 1=weak, 2=moderate and 3 - strong. Staining score was classified as 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). Then both scores were multiplied and patients were divided into two groups: 0-6= low expression group and 7-12: high expression group.

**STATISTICAL ANALYSIS**

Categorical data were formulated as count and percentage. Chi-square test was used to describe the association of these data. Alternatively, Fisher exact test was used if there is 25% of cells less than expected count. Numerical data were described as mean, and standard deviation. Independent sample t-test used for comparison between two groups. The lower level of accepted statistically significant difference is bellow or equal to 0.05, all statistical analysis was done using Graph Pad software Prism 7 * Mac OSX version.

**RESULTS**

The results in table I showed age of patients and control, the mean age in ulcerative colitis patients was 34 years, while in controls - 37.11 years old. However, they were none statically different (p=0.154). There were more representatives of females in the study: 26 (52%) in ulcerative colitis group, and 15 (50%) among controls. The results showed none statistically significant difference in the frequency of sex types among study groups (table I).

**NLRP3 INFLAMMASOME IS UPREGULATED IN ULCERATIVE COLITIS MUCOSA**

The results in table II describes the NLRP3 protein expression among study groups, in terms of relative percentage, intensity score, expression score and combined score. It was found that NLRP3 over-expressed in ulcerative colitis (39.31±9.32), while, in controls were low (14.1±6.97) p<0.001. It was noted that intensity score was almost moderate to high in ulcerative colitis, while being weak and negative in controls (p<0.001). In addition to that, higher immunoreactivity scores were prevalent in ulcerative colitis in contrast to controls the lower scores were reported. The combined score (combined intensity and expression score) was high among 28 (56.00%) in ulcerative colitis, while it was inversely reported (lower expression) in controls 4/30 (13.33%). Furthermore, in ulcerative colitis patients with left sided lesions (49.4%) there was higher percentage of expression than in those with extensive colitis (38.9%) p=0.038.

NLRP3 protein expression localized polyclonal rabbit anti-Cryopyrin (orb182473) diluted as 6 pg/ml, visu-
alized by Super Sensitive peroxidase Detection System Kit (orb219874) A-C, Showing cytoplasmic staining of glandular epithelium and dark cytoplasmic and nuclear staining of infiltrated inflammatory cells D. Diffuse cytoplasmic staining between glands, original magnification (400X) (fig 1).

DISCUSSION
Its widely accepted that inflammasome activation is a prominent feature in inflammatory bowel disease [10]. In this study, NLRP3 protein was expressed in the majority of ulcerative colitis and controls suggesting an active role of inflammasome protein in the pathogenesis of disease. In argument with our results, several human studies highlighted the association of certain genetic polymorphism(s) in NALP3 and CARD8 with UC such as: rs35829419 (Q705K) and rs10754558 as a risk factor for developments of UC leading to elevation of IL-18 and T-helper 1 inflammation in colonic mucosa of inflamed area [11-14]. Pathogenic role of IL-1b have been reported in inflammatory bowel disease suggesting its possible therapeutic target [15]. Furthermore, study by Liu, et al., in 2017 suggested an abnormal activation in NLRP3 protein that plays an important pathogenic role in chronic colitis IL-10 mice and humans [5]. It was found that NLRP3 protein higher expression is found in active disease and patients with extra-intestinal complications. This finding was originally reported in the current study, suggesting a predictive role of worse outcome in patients [5]. It’s argued by Ranson, et al., who in 2018 also reported upregulation of NLRP3 expression in active form of IBD [16]. Further details observed via exploring role of IL-18 and IL-1b in Nlrp3–/– mice is protected in the acute DSS colitis model [17]. The current study highlighted the importance of NLRP3 targeting as a therapy for IBD patients. Study by Bauer et al., suggested involvement of inhibiting NF-κB activation and decreasing mitochondrial reactive oxygen species. This supports our recommendation that future targeting of inflammasome will help to reduce inflammation and its development into sever and poor outcome of disease.

CONCLUSIONS
The NLRP3 protein expression pattern was upregulated among various cellular compartments among ulcerative colitis and correlated with disease activity.

REFERENCES

Author greatly appreciates Dr. Haider F. Ghazi for donation of samples of this study.

ORCID and contributionship:
Manar Mustafa: 0000-0002-6008-8273

Conflict of interest:
The Author declare no conflict of interest.

CORRESPONDING AUTHOR
Manar Mustafa
Middle Technical University
8998-QH, Baghdad, Iraq
e-mail: manar83mostafa@yahoo.com

Received: 08.09.2021
Accepted: 18.01.2022

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article.