Naser E. khudhur et al /J. Pharm. Sci. & Res. Vol. 11(2), 2019, 440-443

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Investigation of lipocalin2 and calprotectin in Ulcerative colitis patients

Naser E. khudhur[1*], Mayada N. Iqbal[1], Samar S. Abd alhussein[2]
[1] *College of Health and Medical Technology, Middle Technical University, Baghdad, Iraq*
[2] *Medical college, Kirkuk university, Iraq*

Abstract

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon. The present study included a total of 75 patients with ulcerative colitis and 35 apparently healthy subjects, all samples were tested by enzyme linked immunosorrbent assay (ELISA).Serum LCN2 concentrations (104.62ng/ml) of ulcerative colitis patients were significantly higher than the healthy control group (49.58ng/ml). The results of Calprotectin in stool revealed that concentrations in ulcerative colitis patients (208.7ng/ml) were higher than healthy controls with significant differences.Serum level of Calprotectin represented that concentrations in ulcerative colitis cases (54.3ng/ml) were higher than healthy controls with significant differences. The severity of disease had significant effect on the concentration of serum levels of LCN2 and Calprotectin in ulcerative colitis patients, but no significant effect of severity on the concentration of fecal Calprotectin

Keywords: lipocalin2, calprotectin, Ulcerative colitis

INTRODUCTION

Ulcerative colitis is an inflammatory disorder of the colon identify by chronic intestinal mucosal inflammation. Unknown causes of aberrant immune response, but hereditary, dietary, and environmental risk factors have playing a role.(1)

Ulcerative colitis can be considered as an immune-mediated disease that affected genetically predisposed individuals because of dysregulated immune responses to the intraluminal antigens (2). bloody diarrhoea and chronic abdominal pain are the main clinical feature of UC (3).

Severity of UC cases classified according to Montreal classification into severe, moderate or mild disease (4)

Ulcerative colitis (UC) and Crohns disease (CD) are diseases of young people with a peak incidence between the ages of teen and forty years. (5, 6) the number of UC patients has increased in developing countries, such as those in Eastern Europe, Asia and Latin America (7, 8)

Neutrophil granulocytes infiltration and mucosal Epithelial cell damage is important, characteristic of local inflammatory process of Ulcerative colitis. Neutrophils granulocytes have two major granules families azurophilic (primary granules) and specific (secondary granules), which are formed at different neutrophils stages of maturation. The specific granules carry many different components, like collagenase, lactoferrin, lipocalins and lysozyme (9)

On stimulation condition, the neutrophils granulocytes can release huge quantity of toxic oxygen radicals and different types of granular proteins also soluble proteins like calprotectin. newly descriptions of Lipocalin 2((LCN2) from secondary granules of human neutrophils cells.LCN2 also called human neutrophil lipocalin (HNL).(10)

LCN2 is a 198 amino acid long secreted glycoproteins which encoded by a gene positioned at the chromosome locus 9q34.11 (11) LCN2 have a strong bacteriostatic protein properties located and stored in neutrophils granules and secreted at inflammatory sites. (12) LCN2 have a role in inflammatory bowel disease (IBD) pathogenesis and have benefits as marker for intestinal mucosal inflammation and correlated with UC activity (13, 14) LCN2 is expressed in various cell types including adipocytes and epithelium of the gastrointestinal, neutrophilic granulocytes and respiratory and urogenital tracts.(15)

Calprotectin is a zinc and Calcium binding heterodimer of 36.5 kDa that related to S100 family .Calprotectin is predominantly located in granules of polynuclear neutrophils (PN) where it forms five percent of total proteins and sixty percent of cytosolic proteins in macrophages and monocytes cells, also Calprotectin expressed at a low concentration at epithelial cells (16).

An antimicrobial property of Calprotectin involves (zinc) Zn2+ ion sequestration resulting that result in decline activity of zinc-dependent enzymes, that's cause inhibition of bacterial cell growth. It's encourage stimulation of monocytes/macrophages ,acting by Toll-like receptor-4(TLR4), activates transcription factors(TF) like nuclear factor-κB(NF-κB),triggering in increased secretion of proinflammatory cytokines(17)and metalloproteinase enzymes(18) , moreover to stimulating interleukin-17-producing T cells , which have a role in UC Pathophysiology(19) so our study designed to investigate the level of lipocalin2 and sera and feacal calprotectin in patients with ulcerative colitis

MATERIALS AND METHODS

A total of 100 patients with ulcerative colitis from both genders (55 males, 45 females) whose ages ranged (19-62 years) were selected. Was administered Azadi teaching hospital in Kirkuk-Iraq and Al-yarmouk hospital in Baghdad-Iraq. Exclusion criteria were: Previous history for colectomy, Pregnancy Patients taken antibiotics or others treatments, other coexisting autoimmune diseases, infection, chronic disease or cancer, other. While control group consisted of 45 supposed healthy subjects (25 males, 20 females) without any history of gastrointestinal or other diseases. Their ages ranged (21-42 years).

Venous blood (5ml) was drawn from patients group and control group .Sera was separated by centrifugation at 3500

rpm for 10 minutes. Also stool samples were taken from both groups. Both separated sera and stool samples were stored in separated plain tubes at -20 C^o before further testing. Stool was collected before sigmoidoscopy since bowel cleaning could affect the results of FC. Each participant was provided with a sterile fecal specimen tube and morning single fecal samples (5-10g) were collected.(20)

Serological estimation by sandwich enzyme linked immune-sorbent assay (ELISA) was done for (Lipocaline 2,calprotectin) .using kits from (Mybiosource) USA company for detection of Lipocaline 2 and calprotectin

RESULTS

The study samples consisted of 100 ulcerative colitis patients of both sexes (55 males and 45 females) and 45 healthy individuals samples as control group (25 males and 20 females)as represented in Table (1) . And all the patients were with active UC disease (100/100). The age ranged between (19-62 years) with mean (38.76 years) of ulcerative colitis patients. While the age for healthy people ranged between (21-42) years with mean (30.9 years). The duration of disease ranged between (1-195 month) with mean (16.2) months

The levels of Lipocalin 2 and calprotectin in studied groups:

The Lipocalin 2 (LCN2) concentration ranged between (10.98-244.62ng/ml) with mean (104.62ng/ml) in ulcerative colitis patients, which significantly higher than healthy control group and ranged between (14.85-74.34 ng/ml) with mean (49.58 ng/ml)

The results showed that the difference between fecal calprotectin with range (94.0-375.6 ng/ml) and mean (208.7 ng/ml), from healthy groups that ranged between (125-194.7ng/ml) with mean (150.8) as represented in table (2),while serum level of calprotectin was range between (10.6-88.6 ng/ml)with mean (54.31ng/ml) in ulcerative colitis patients, while the Calprotectin concentration in healthy people ranged between (28.4-47.3 ng/ml) with mean(39.0 ng/ml) as represented in table (2).The severity had no significant effect on the concentration of fecal Calprotectin in UC patients (p=0.632).while serum Calprotectin reveal significant differences in serum level according to severity of disease (p=0.010).also LCN2concentration showed that the severity has significant effect on the levels of LCN2 in UC patients (p=0.005). As revealed in table (3)

Table 1: characteristics of ulcerative colitis patients and control groups.

Variable	Ulcerative colitis patients	Healthy Control
No. (male/ female)	100 (55/45)	45 (25/20)
Age range (mean)years	19-62 (38.76)	21-42(30.90)
Age group having UC no.,%	>40(42) <40(58)	0
Severity no.,% (mild ,moderate ,sever) NO.	21(21%), 38(38%), 41(41%)	0

Table 2: The levels of sera LCN2 and fecal calprotectin in studied groups

Parameter ng/ml	Study groups	No	Mean (range)	Std.error	t-test (p-value)
LCN2	Patient group	100	104.62(10.98-244.6)	7.43	0.005
	Healthy Control group	45	49.58(14.85-74.34)	3.58	
Serum calprotectin	Patient group	100	54.3(10.6-88.6)	5.08	0.010
	Healthy Control group	45	39.0(28.4-47.3)	2.12	
Fecal calprotectin	Patient group	75	208.7 (94.0-375.6)	12.84	0.005
	Healthy Control group	35	150.8(125-194.7)	6.98	
Significant at P<0.05					

Table 3: The level of Lipocalin2 and Calprotectin in patients group according to severity

Groups / Parameter	Ulcerative colitis patients=100			ANOVA (p-value)
	Mild (N=16)	Moderate (N=28)	Severe (N=31)	
LCN2 ng/ml				0.005*
Range	16.83-89.10	22.23-169.38	10.98-244.62	
Mean	58.87	98.70	125.42	
Standard error	10.70	9.49	11.81	
Serum calprotectin ng/ml				0.010*
Range	59-61	10-75	43-88	
Mean	60	41	72	
Standard error	1.03	6.17	6.40	
Fecal calprotectin ng/ml				0.632*
Range	175-205	94-375	122-295	
Mean	188	198	221	
Standard error	9.00	23.52	17.60	
*Significant at P<0.05				

Table 4: Correlation between studied parameters among ulcerative colitis patients

Parameter		Serum calprotectin (ng/ml)	Fecal calprotectin (ng/ml)
Lipocalin 2 (ng/ml)	Pearson Correlation	.104	.063
	Sig. (2-tailed)	.647	.741
Serum calprotectin (ng/ml)	Pearson Correlation		-.208
	Sig. (2-tailed)		.352
***Significant at P<0.05**			

Table 5: Cuts-off values, sensitivity and specificity of LCN2 and fecal and serum calprotectin in the differentiation of UCs patients from controls.

Parameter	Cuts-off values	Sensitivity (%)	Specificity (%)	AUROCs (95% CIs)	Ps-values
LCN2	65.61 ng/ml	88	80	0.949	0.002
Serum calprotectin	41.08 ng/ml	82	67	0.773	0.005
Fecal calprotectin	163.35 ng/ml	80	75	0.649	0.010

Correlation between studied parameters among Ulcerative colitis patients:

The study revealed significant positive correlation between serum levels of lipocaline2, fecal and serum calprotectin in UC patients. But there was no correlation between calprotectin in blood with stool as represented in Table (4)

Generalized linear model for outcome prediction:

By using the receiver operating characteristic (ROC) curve, table (5) show that LCN2 and serum and fecal calprotectin could differentiate UC patients from control groups as well as the cut-off values. The LCN2cuts-offs values of 65.61ng/ml had a sensitivity of 88% and specificity of 80% with the AUROCs of 0.551(P value = 0.002). While Serum calprotectin cuts-off values of 41.08 ng/ml had a sensitivity of 82% and specificity of 67%, at AUROC of 0.773 (P value = 0 .010).Also Fecal calprotectin cuts-off values of 163.35 ng/ml had a sensitivity of 80% and specificity of 75%, with the AUROCs of 0.649(P value = 0.005)

DISCUSSION

Migration of Neutrophils to the colonic mucosal layer is a hallmark of IBD (21)

In our study, Serum lipocaline2 levels in the UC patient group were found to be significantly higher than in the control group with (p=0.005)

Although this study was being conducted, Oikonomou et al. (14) demonstrated similar results with the same methodology.

In their study, and the levels of serum lipocaline2 in the UC cases group were found to be significantly elevated levels than control groups. LCN2 believed as specific markers of neutrophils activation, and increased in the areas of local inflammation and its reflects the size of inflammatory response (22). also others researchers were able to show that serum lipocalin 2 is a strong UC activity biomarker, with greater sensitivity than white blood cell count(WBC) or CRP(23, 24).Strong expression of LCN2 from Both mucus of goblet cells and cytoplasm of enterocyte suggest that Lipocalin2 is secreted from goblet cells is an major mechanism for LCN2 secreted in to gut lumen. Additionally LCN2 may leak into the lumen of the gut from neutrophils cells .this has a an importance in clinical testing.and fecal lipocalin 2 correlated with UC severity during intestinal inflammation (25) .

Furthermore serum calprotectin have short half-life about five hours, it may allow, a more accurate diagnostic test of inflammation condition compared with available inflammatory markers (half-life of albumin 19 days, CRP 18 h,) (26). In IBD cases, serum calprotectin levels may reflect the released calprotectin from immune cells such as from activated neutrophils cells, macrophages, monocytes and epithelial cells. Serum calprotectin reveal a strong correlation with other inflammatory parameters (27, 28)

Fecal Calprotectin level in our study were higher than control group with significant (p=0.005), FC levels are largely elevated in severe UC.(29)In agreement with others studies fecal calprotectin directly proportional to neutrophil cells infiltration in the GIT mucosa (30)Many researches show that Calprotectin test is highly specific and sensitive for the evaluation of UC activity and for relapse in IBD (31). Irritable bowel syndrome (IBS) differentiated from IBD by using calprotectin test (32) Others represent that FC levels are dramatically elevated in severe UC (29).The levels of fecal calprotectin are directly proportional to neutrophil infiltration in the gastrointestinal tract (GIT). (33)

Currently, the level of calprotectin in stool is believed to be the best noninvasive marker of inflammation of intestinal mucosa. Calprotectin protein is stored in neutrophils in some extent in monocytes, thus reflects intestinal mucosal infiltration and shedding. Lipocalin2 is released from both neutrophils and activated epithelial cells it has the potentiality to be sensitive UC activity marker than calprotectin, especially in chronic inflammation conditions where neutrophils are abundant (34)

CONCLUSION:

Results of current study showed that Lipocalin2 and serum calprotectin may play important roles in UC severity monitoring.

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