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RESEARCH ARTICLE

Serum levels of IL-13 and PAF in ulcerative colitis patients

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Abstract

Background: Interleukin (IL) IL-13 and Platelet activating factor (PAF) can be the key roles that influence the cause of colonic mucosa inflammation and ulceration in UC patients also platelets are new players in the mucosa of colon. **Objective**: the study aimed to investigate the sera IL-13 and PAF levels in patients with ulcerative colitis and normal controls and define if these markers levels differences to get with the severity and extent of disease according to endoscopic findings **Patients and methods:** The present study, extend from December 2014 and May 2015, a total of 75 patients with ulcerative colitis from both sex (40males,35females) and their ages ranged between (16-62 years). Other of 25 apparently healthy subjects (16 males, 9 females) without any history of gastrointestinal or other diseases. Their ages ranged between (19-54 years).All samples were tested by Enzyme Linked Immune Sorbent Assay (ELISA) for IL-13, PAF and by automated device for complete blood count. The suitable methods were used in order to analyze and assess the results. **Results:** Present study **showed** high concentrations of IL-13 with mean (683.850pg/ml) of ulcerative colitis patients contrasted with healthy control (87.200 pg/ml)**.**In spite of this the severity and the disease extent have no significance influenced with the concentration differences of IL-13in UC patients. The PAF concentrations ranged between (0.00-234ng/ml) with mean (23.093) of ulcerative colitis patients, the severe UC patients group showed the higher percentage for PAF concentration comparing to mild and moderate but this difference has no significance at p=0.510 . PAF concentration was positive in 37.5% (distal/ left-sided colitis),18.75% (extensive colitis),18.75% (proctitis) and 25% (proctosigmoditis) but this differences no significance in UC patients (p=0.497).The statistical analysis results of complete blood count showed significant differences in Monocytes , Eosinophil , Basophil , red blood cells and Hb ($p<0.05$) between the patients group and the control group ,While Neutrophil **,** lymphocyte and platelets results showed no significant ($p>=0.05$). IL-13 results showed high significance correlation with platelets count.

Introduction:-

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon (1).Patient shave choppy disease flares interspersed with absolution periods (2). According to the Montreal classification, the UC extent can be classified as :Ulcerative proctitis Involvement limited to the rectum (that is, proximal extent of inflammation is distal to the rectosigmoid junction), Left sided UC (distal UC) Involvement limited to a proportion of the colorectum distal to the

splenic flexure and Extensive UC (pancolitis) Involvement extends proximal to the splenic flexure (3). The cause of UC is unknown. UC is associated with a Th2-atypical response, Ulcerative colitis shows a particular immune pathway; in the past, it was thought to be a Th2 dominant disease (4). Th2 cells output IL-4, IL-5, IL-6, IL-10, and IL- 13 and stimulate atopy through activation of mast cells and induction of IgE responses (5).IL-13 has become the leading candidate effector cytokine driving inflammation in ulcerative colitis based on data from a murine model of UC (6).The excessive production of IL-13 in ulcerative colitis is thought to lead its deleterious effects, particularly as IL-13 has demonstrated toxic effects on colonic epithelial cells and the epithelial barrier. IL-13 activates the proapoptotic molecule caspase 3 in mouse colonic epithelial cells, a process involving the tumor necrosis factor super family cytokine as well as tumor necrosis factor α itself, all proteins found to have increased expression in patients with active ulcerative colitis (7).

Moreover, recent investigations appeared that the high blood coagulative state in patients with UC was closely related with the abnormal function of platelets, which resulted in high a probability of microvascular thrombosis and microcirculation dysfunction, main causative factors for UC.(8) Platelet-activating factor (PAF) is a potent ulcerogenic agent in the gastrointestinal tract The role of this mediator, especially in connection with the unknown etiology of chronic inflammatory bowel diseases, remains poorly understood. .(9) PAF related to increase prostaglandin E2 production and chloride ion secretion by isolated human intestinal mucosa .Increased levels of PAF are produced within inflamed mucosa of patients with UC or Crohn'sdisease (10,11). PAF may play an important role in inducing intestinal damage during the course of ischemia-reperfusion injury and neonatal necrotizing enterocolitis (12).

Patients and methods:-

A total of 50 patients with ulcerative colitis from both sex (40males,35females) and their ages ranged (16-62 years). A structure interview using standard questionnaire was administered by interviewers with patients at their visit to Al-yarmouk hospital in Baghdad-Iraq and Al-Imameen medical city. During the period from December 2014 and May 2015.Exclusion criteria were: Pregnancy, previous history for colonectomy, antibiotic use when patients were admitted to the hospital and previous history of medical treatment. , Other concurrent infection, chronic disease or cancer. The control group consisted of 25 apparently healthy subjects (16 males, 9 females) without any history of gastrointestinal or other diseases. Their ages ranged (19-54 years).

Specimen: Venous blood samples (5 ml) from patients and controls were collected (1ml of sample) in EDTA tube for CBC test and the 4ml of sample in dry clean plain tubes. Serum was obtained from freshly drawn blood. Serum was quickly frozen at -20^{o}C and stored until the tests were processed.

Study Protocols: (Heamatological estimation for complete blood count (CBC) & Serological estimation by enzyme linked immune-sorbent assay (ELISA) was done for (IL-13, PAF).

Materials: two serological tests were performed in this study including Enzyme immunoassay technique kit for detection of IL-13and PAF in serum (Cusabio _China).**Statistical Analysis:** By using SPSS version (16.0)**,** the following statistical data analysis approaches were used in order to analyze and assess the results of the study:

1. **Descriptive data analysis**:
2. **Inferential data analysis:**

These were used to accept or reject the statistical hypotheses, which included the following :Chi-square (X^2) and Student test (t-test). Logistic correlation was used to study the correlations between factors.

Results:-

Demographic characteristics of study groups appeared the age distributions and data of the patients were summarized in table (1).The age ranged between (16-62 years) with mean (36.525) of ulcerative colitis patients. While the age for healthy people ranged between (19-54) years with mean (30.2) .the duration of disease ranged between (1-204 month) with mean (19.400).The results was with no significance (p=0.053). When employment category was taken into consideration skilled manual showed more positive results 46/75 (62%) than unskilled 12/50 (16%) , housewives 11/50 (14%) and students 6/50 (8%) ,While the control group showed 15/25 (60%) skilled ,2/25 (8%) unskilled ,2/25(8%) housewives and 6/25(24%) as students. the results was highly significance (p=0.041). Regarding to the smoking, the nonsmoker patients 41/75(54%) noted being while the current smokers 24/75 (32%) and ex-smokers10/75(14%).These results were with highly significance (p=0.002).

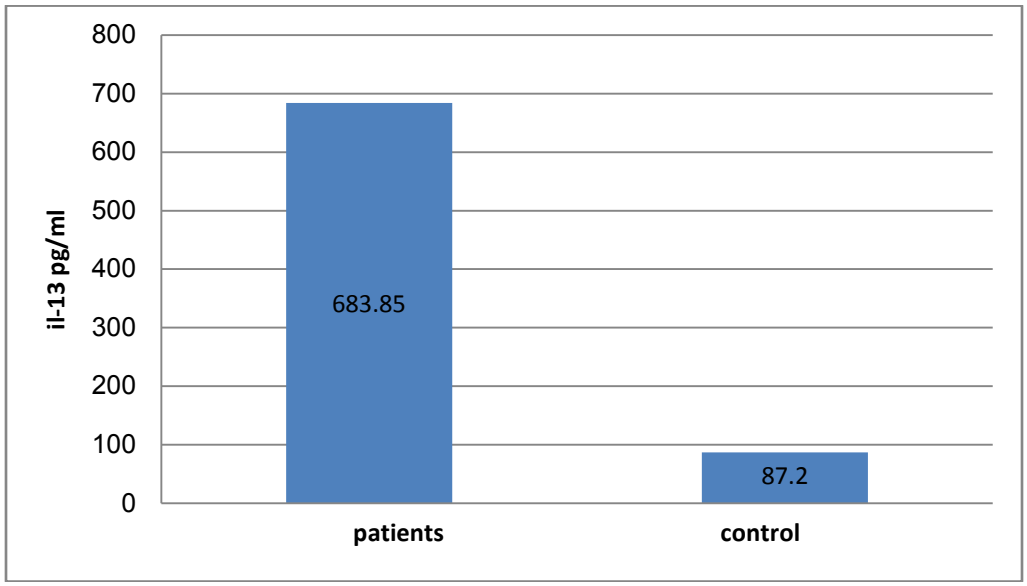
The levels of IL-13 in studied groups:

Table and figure (1) represented the most common statistics for studying and analyzing the studied parameter of IL-13 in the studied groups, such as mean, standard deviation, standard error, minimum and maximum for estimation (range) in each group. The IL-13 concentration ranged between (.00 -3500 pg/ml) with mean (683.850) of ulcerative colitis patients. While, IL-13 concentrations of healthy people ranged were between (.0-609 pg/ml) with means (87.200).

Table (1) the levels of IL-13 in studied groups

IL-13 pg/ml	N	Mean (range)	Std. deviation	Std. error	t-test (p-value)
Patients group	**75**	**683.850 (0-3500)**	**723.983**	**114.471**	**0.001**
Healthy Control group	**25**	**87.200 (0-609)**	**135.581**	**30.316**	

Significant at p<0.05



The figure (1) represents the levels of IL-13 in studied groups

The serum level of IL-13 was studied according to disease severity, the results showed higher percentage of seropositivityof IL-13 in severe patients group but with no significantly. (p= 0.638). Table (2)

Table (2) the percent of IL-13 seropositive in patients group according to severity

Groups Results			Severity			Total
			Mild	Moderate	Severe	
	+ve	% within IL-13 pg/ml	10%	30%	60.0%	100.0 %
	-ve	% within IL-13 pg/ml	.0%	30.0%	70.0%	100.0 %
Total		% within IL-13 pg/ml	8%	30.0%	62.0%	100.0 %
p= 0.638						

Significant at p<0.05

According to disease extent, IL-13 concentration was positive in 60 cases, there were 40.0% for distal/ left-sided colitis, 17.5% in extensive colitis, proctitis and proctosigmoditis27.5% and 15.0% respectively. The concentration of IL-13 has no significance differences according to the extent patients (p=0.767).table (3)

Table (3) the percent of IL-13 seropositive in studied patients group according to disease extent

Groups Results			Extent				Total
			distal/left-sided colitis	extensive colitis	Proctitis	Proctosig moditis	
IL-13 pg/ml	+ve	% within IL-13 pg/ml	40.0%	17.5%	27.5%	15.0%	100.0%
	--ve	% within IL-13 pg/ml	40.0%	.0%	40.0%	20.0%	100.0%
Total		% within IL-13 pg/ml	40.0%	14.0%	30.0%	16.0%	100.0%
p=0.767							

Significant at p<0.05

The levels of PAF in studied groups :

The PAF concentration ranged between (0.00-234ng/ml) with mean (23.093ng/ml) of ulcerative colitis patients. While healthy people appeared lower concentrations between (0-20 ng/ml) with mean (2.235ng/ml). PAF levels significant higher in UC group than these observed in the normal subjects the details showed in table (4)

Table (4) the levels of PAF in studied groups

PAF ng/ml	N	Mean (range)	Std.deviation	Std.error	t-test (p-value)
Patient group	75	23.093(0-234)	55.251	8.736	0.023
Healthy Control group	25	2.235(0-20)	4.371	0.977	

Significant at p<0.05

The results showed that the levels of PAF has no significance affected by the severity of disease among patients (p=0.510). PAF concentrations were positive in 24 cases which they 0%, 37.5% and 62.5% for mild ,moderate and severe respectively, table (5). Also PAF concentrations were showed identical results according to the extent of UC disease positive in 24 cases, there were 37.5%(distal/ left-sided colitis),18.75% (extensive colitis ,18.75% (proctitis) and 25% (proctosigmoditis) ,the extent has no significance with the concentration of PAF in UC patients (p=0.497).

Table (5) The percent of PAF seropositive in patients group according to severity

Groups Results			Severity			Total
			Mild	Moderate	Severe	
PAFng/ml	+ve	% within PAF	.0%	37.5%	62.5%	100.0%
	-ve	% within PAF	11.8%	29.4%	58.8%	100.0%
Total		% within PAF	8.0%	32.0%	60.0%	100.0%
p=0.049						

*Significant at p<0.05

Table (6) The percent of PAF seropositive in studied patients group according to disease extent

Groups Results			Extent				Total
			distal/left-sided colitis	extensive colitis	proctitis	proctosig moditis	
PAF ng/ml	+ve	% within PAF	37.5%	18.75%	18.75%	25%	100.0%
	-ve	% within PAF	41.3%	14.7%	32.3%	11.7%	100.0%
Total		% within PAF	40.0%	16.0%	28.0%	16.0%	100.0%
p=0.497							

Significant at p<0.05

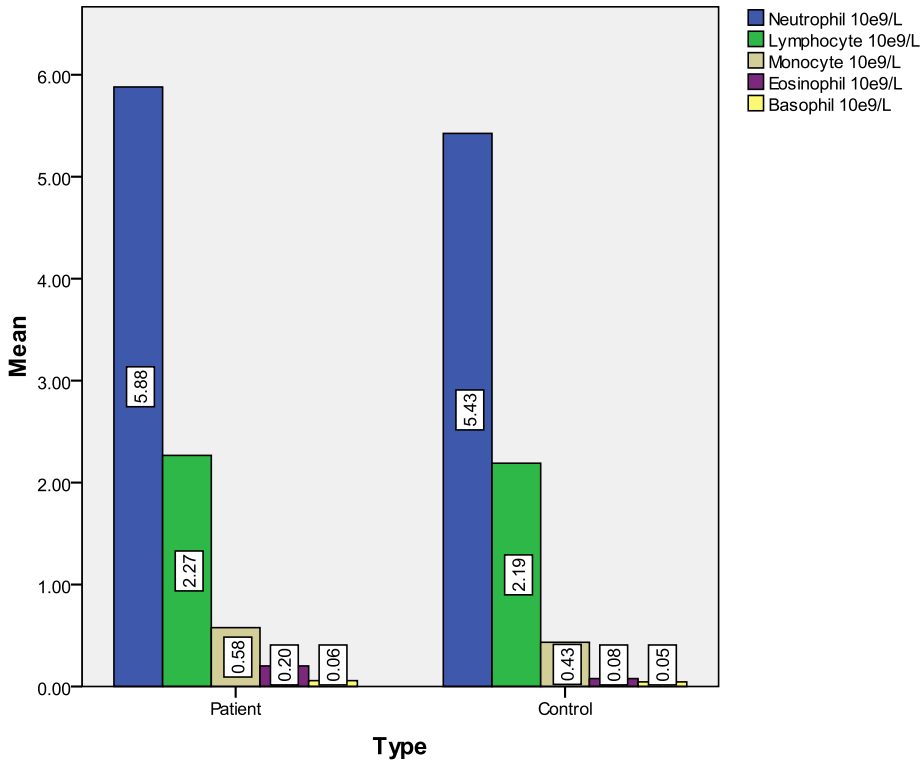
Complete blood picture:

The statistical analysis results of complete blood picture showed that there were significant differences in Monocytes , Eosinophil , Basophil , RBC and Hb (p<0.05) between the patient group and the control group . While Neutrophil, lymphocyte and platelets results showed no significant (p>=0.05).

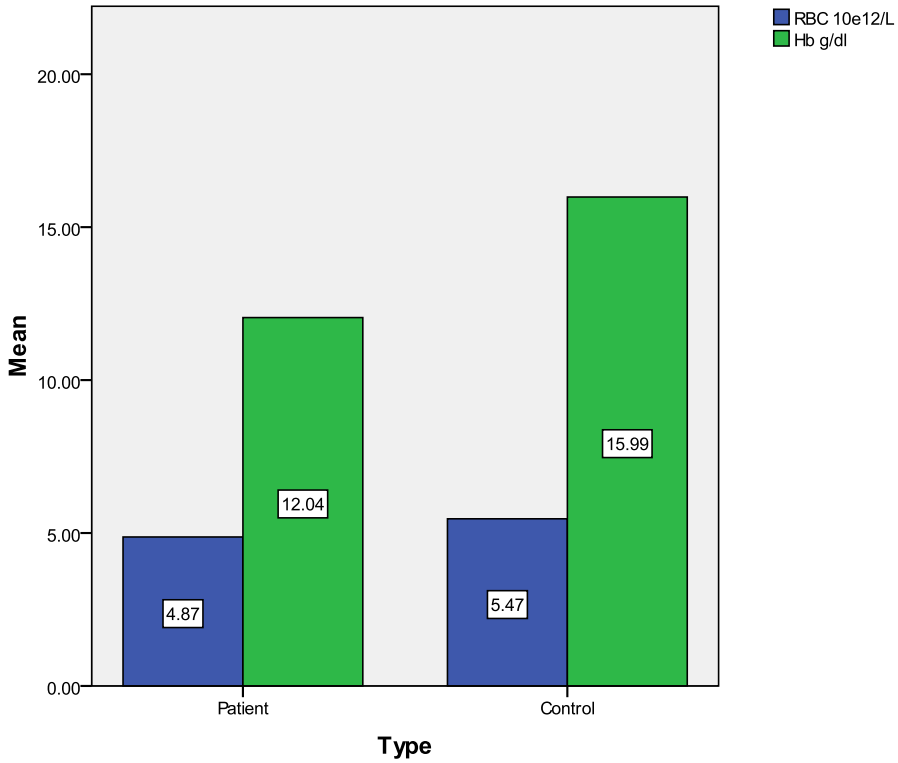
Table (4-17) The levels of complete blood picture in studied groups.

Groups / parameters		Patients	control	t-test (p-value)
Neutrophil 10e9/L	Mean	5.8800	5.4250	0.053
	range	(4.10-8.10)	(3.90-6.80)	
	Std. Error	0.13550	0.18004	
	Std. Deviation	0.85698	0.80516	
Lymphocyte 10e9/L	Mean	2.2667	2.1900	0.520
	range	(1.08-3.20)	(1.10-2.70)	
	Std. Error	0.06913	0.09484	
	Std. Deviation	43724.0	0.42414	
Monocyte 10e9/L	Mean	0.5772	0.4337	0.001
	range	(0.25-0.80)	(0.24-0.73)	
	Std. Error	0.02341	0.03512	
	Std. Deviation	0.14808	0.15704	
Eosinophil 10e9/L	Mean	0.2017	0.0777	0.001
	range	(0.02-0.84)	(0.03-0.15)	
	Std. Error	0.02576	0.00701	
	Std. Deviation	0.16290	0.03136	
Basophil 10e9/L	Mean	0.0575	0.0452	0.049
	range	(0.02-0.10)	(0.01-0.08)	
	Std. Error	0.00284	0.00528	
	Std. Deviation	0.01795	0.02359	
RBC 10e12/L	Mean	4.8708	5.4650	0.004
	range	(3.10-6.20)	(5.10-5.90)	
	Std. Error	0.13653	0.05345	
	Std. Deviation	0.86349	0.23902	
Hb g/dl	Mean	12.0438	15.9850	0.000
	range	(8-16.40)	(14.30-17.70)	
	Std. Error	0.31404	0.16243	
	Std. Deviation	1.98614	0.72640	
Platelet 10e9/L	Mean	310.6550	328.3000	0.414
	range	(189.00-510.00)	(211.00-401.00)	
	Std. Error	13.58942	13.28297	
	Std. Deviation	85.94704	59.40326	

Significant at $p<0.05$



The figure (2) the number of WBC in studied groups



The figure (3) The mean of RBC and Hb in studied groups

Correlation between studied parameters among ulcerative colitis patients:

IL-13 levels appeared high significance opposite correlation with platelets count (r = -.327, p = .039). Other results of parameters showed no significance correlation (table 7).

Table (7) Correlation between studied parameters among ulcerative colitis patient

		IL-13 pg/ml	PAF ng/ml
IL-13 pg/ml	**Pearson Correlation**	**1**	**-.262**
	Sig. (2-tailed)	**---**	**.103**
PAFng/ml	**Pearson Correlation**	**-.262**	**1**
	Sig. (2-tailed)	**.103**	**---**
Neutrophil 10e9/L	**Pearson Correlation**	**-.187**	**.068**
	Sig. (2-tailed)	**.249**	**.677**
Lymphocyte 10e9/L	**Pearson Correlation**	**.016**	**-.167**
	Sig. (2-tailed)	**.924**	**.303**
Monocyte 10e9/L	**Pearson Correlation**	**-.089**	**.122**
	Sig. (2-tailed)	**.584**	**.454**
Eosinophil 10e9/L	**Pearson Correlation**	**.136**	**.144**
	Sig. (2-tailed)	**.403**	**.375**
Basophil 10e9/L	**Pearson Correlation**	**.005**	**-.001**
	Sig. (2-tailed)	**.975**	**.994**
RBC 10e12/L	**Pearson Correlation**	**-.126**	**.049**
	Sig. (2-tailed)	**.440**	**.762**
Hb g/dl	**Pearson Correlation**	**-.089**	**-.110**
	Sig. (2-tailed)	**.587**	**.501**
Platelet 10e9/L	**Pearson Correlation**	**-.327***	**-.051**
	Sig. (2-tailed)	**.039**	**.752**

Significant at p<0.05

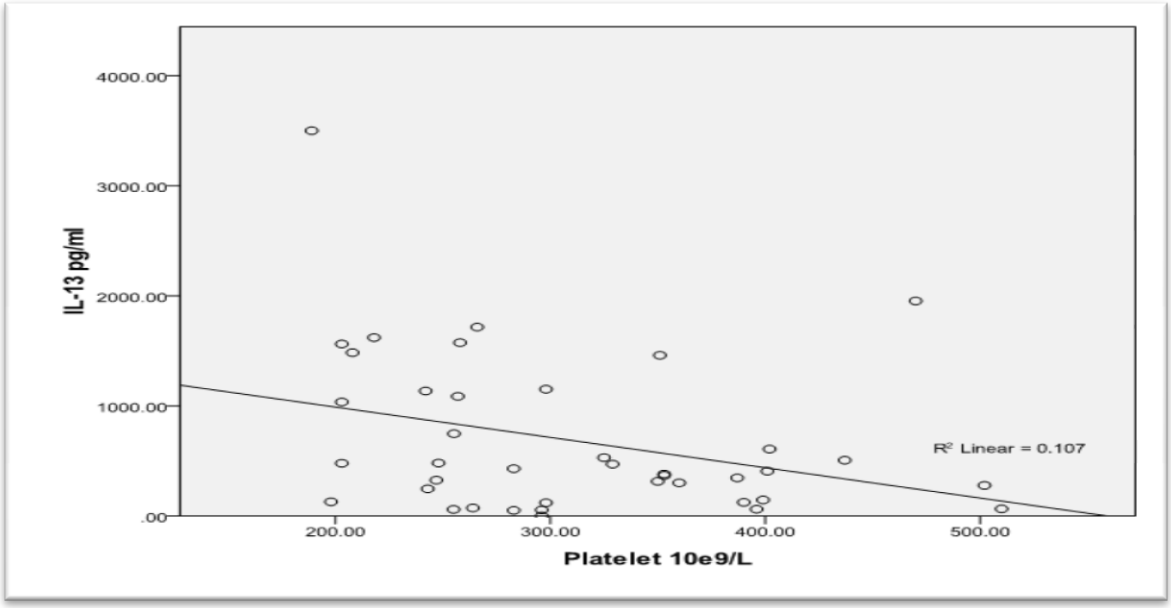


Figure (4) correlation between serum level of IL-13 and number of platelet

Discussion:-

Ulcerative colitis is a chronic, relapsing and progressive inflammatory bowel disease (IBD) of uncertain etiology thought to be stimulated by genetic ,environmental, and immunological factors .(13)This disease results from an aberrant immune response and loss of tolerance to many self-antigens, leading to chronic inflammation of the gut.(14)The study samples consisted of 40 males, 35 females so there is a slight appearance in male rather than female and the age ranged between (16-62 years) with mean (36.525) of UC patients. Disease prevalence was high (58.6%) in patients between 18 to 35 years of age, there was no such difference in the prevalence found among the patients with ages ranged (36 - 55 years) and (56 - 80 years) (24.4% VS 17.2%).(15) Earlier studies showed that men and women were equally affected which is matched those reported that UC is most common in 15 and 40 years of age (15), and the male to female ratio was 1.2 for UC. The mean duration of disease was found to be 9.7 (SD 6.4) years for UC (p=0.815) (16).Moreover this study showed the higher incidence of ulcerative colitis among males (54%) than females (46%) within the patient's group in consistency with other previous (17) whereas in other studies represent a higher incidence rate among females than males (18).However, most North American studies show that UC is more common in males than females (17).

Present study disagrees with other earlier studies which showed the mean age of the patients with UC was 36.61±13.06 (min: 16, max: 74) years and there was no statistically significant difference between the groups regarding age and gender. (19)

The variation in the age incidence and gender reflects the interaction of both environmental and genetic factors in different racial, social and geographical areas in the world also hormonal changes during adolescence (20).The study documented the duration of disease ranged between (1-204 month)with mean (19.400) it agrees with different studies with a mean of 8-13.9 months and appeared to have lowered in recently published articles (21) and the mean disease duration in all the patients was 12 years (95% CI: 9–15) (22). Furthermore it is disagreed with other researches; The interval between the onset of symptoms and the diagnosis of chronic UC ranged between 10 days and 10 years (mean 2 years) (23). The never smoker patients showed more positive results (54%) than the current smokers (32%) and ex-smokers (14%).Smoking may modify the phenotype, it protects against ulcerative colitis but increase risk of Crohn's disease (24).Other studies, there was an inverse relation between smoking and the presence of UC ($p \leq 0.002$ and $p<0.0001$); cessation of smoking was accompanied by appearance of disease. (25) It is reported that nonsmokers have a higher risk of developing UC (26). Association between ulcerative colitis and smoking was first described in 1982 by Logan and colleagues. They noted that 8% of patients were smokers compared to 44% of control patients (27).Previous studies were varied the proportions of smokers than nonsmokers, in such study showed that percentage of UC patients who never smoke was 45% (18), while in another one, it was 79% (25).Anyway, smoking have been shown to be positively associated with CD, but negatively associated with

UC .These relationships are the most firmly established epidemiological factors with these two conditions. The first association of non-smoking with UC was made over 30 years ago (28). In one research, childhood exposure to environmental tobacco smoke reduced the risk of developing UC in adulthood (29). Motley *et al*, study found that 107 (70%) developed ulcerative colitis after cessation with a peak in the first year and 52% in the first three years after cessation. Such event revealed that when the patients stopped smoking it can be in relation to the onset of UC. Smoking influence cellular (30) also humoral immunity (31). Alterations in regulatory T cells have been described with heavy smokers having a high level of suppressor T cells, and low ratio of CD4+ to CD8+ T cells. These changes, which induce immune suppression, revert to normal on cessation of smoking (30). Patients with heavy smoke may have decreased levels of IgA in both saliva (31) and secretion of intestine. (32). In addition , the study agrees with other researcher that concluded that active smokers were less likely to develop UC compared to individuals who were never smokers or ex-smokers.(33).This study observed that disease extent was distal/left sided colitis the highest 38% ,extensive 16 % ,proctitis 30% , proctosigmoditis 16% **.** It is matched with earlier study that says disease extension was assessed in all studies. In the common forms, UC seemed to be limited to the left side of the colon .Proctitis was the second most common form of UC. In the least common form, UC extended beyond the splenic flexure. (25) It is also showed by other researcher that a total of 156 patients (42%) had distal colitis (distal to the splenic flexure), 82 (22%) had substantial colitis (distal to the hepatic flexure) and 104 (28%) had total colitis (21).Furthermore, Colitis extent was Proctitis 8 (3%), Left-sided colitis 73 (32%), Extensive colitis 148 (65%) (20), and according to UC extent, 73 (46.5%) had pancolitis, 60 (38.2%) had left-sided colitis and 24 (15.3%) had proctosigmoiditits (15).The extent of UC in this study was different from that reported by Al –Akashi & Shubbar which showed that the corresponding result were proctitis 31.7% , proctosigmoditis 15.6% , subtotal colitis 23% and pancolits 30% (34).According to Truelove and Witts' criteria, mild, moderate and severe disease activity was determined in 12 (26.1%), 23 (27.1%) and 11 (12.9%) patients, respectively. (15) In this study the proportion of patients with UC was (mild 10 %, moderate 30 %, sever 60%) agrees with other research that reported UC was moderate 95, and severe UC was 62 (15), and Chronic UC was mild in 203 patients (55%), moderate in 136 (37%), and severe in 33 (9%) (23). Present study revealed a significant increase in the concentration of IL-13 in the serum of UC patients which agrees with other studies that achieved significantly higher concentrations of IL-13, IL-17 in the serum of patients with UC, compared to the control group. (35)

On the basis of results obtained from studies of the ulcerative colitis model, some of the few models to exhibit a TH2 profile, , CD1d, which presents lipid rather than protein antigens to T cells, these unique observations could explain some of the discrepancies of past studies, but will require confirmation before being accepted. If true, obstruction of IL-13 could be responsible for an exciting new approach to ulcerative colitis treatment. (36) Moreover, all IL-13 values of patients were increased compared to the control values, emphasizing the highest ones in the moderate activity subgroup (37,38).Cytokines surely have importance because they are directly responsible for mucosal injury (39). In UC patients, the levels of spontaneous IL-13 production were similar to the levels observed in the control group; however, after the stimulation, the cytokine concentration became significantly higher than in controls (40). Mucosal inflammation is almost always mediated by one of two pathways: either an excessive Th1 response that is associated with increased secretion of IL-12, gamma interferon and TNF or an excessive Th2 response that is associated with increased secretion of IL-4, IL-5 and IL-13 (41).In the mucosa of intestine from patients with UC, the expression of pro-inflammatory cytokines was significantly raised. Future investigations will show the impairments of cytokine network for UC beginning and progression. Serum cytokines measurement has not correlated well with the activity (42).PAF is a phospholipid mediator that has been implicated in the pathology of NEC, IBD, and asthma (43, 44). The current study showed that PAF concentration ranged between (0.00-234ng/ml) with mean (23.093) of ulcerative colitis patients. Biopsy samples of colonic and ileal mucosa from IBD patients contain increased amounts of PAF, PAF pro-inflammatory effects can play critical roles in the pathogenesis of IBD and conceder it the key for intervention in chronic inflammatory diseases of the intestine. (45) PAF causes injury to intestine initially by induction of an inflammatory cascade; however, the mechanisms of this pathway are not fully understood (46).PAF levels were increased in inflamed mucosa of patients with ulcerative colitis or Crohn's disease (47). A fact brought to light that PAF can directly damage epithelial cells of intestine by activating chloride channels which lead to intracellular acidosis and apoptosis (46). Releases of PAF could contribute to the inflammatory process in several ways. PAF has been shown to stimulate neutrophil aggregation and degranulation, leading to enhanced release of other pro-inflammatory agents, such as leukotriene B4, as well as tissue-damaging free radicalslysosomal enzymes (48). It is thus suggested that platelet-activating factor may be involved in the pathogenesis of the inflammatory response in ulcerative colitis and that its inhibition by steroids, 5-aminosalicylic acid, and salazopyrine may be an additional mechanism to explain their therapeutic effects. Whether or not agents that inhibit PAF synthesis might also be effective in reducing damage in human inflammatory bowel disease is not yet known.

Statistical analyses of WBC and RBC showed no significance differences. The Hb concentration results showed some abnormality, so there is a high significance. As it was mentioned earlier that CBC include these results : anemia (hb<14 g/dl in male and <12 g/dl in female) and thrombocytosis platelet count >350,000/ul (49,50) also WBC levels were statistically insignificant in comparing patients and controls (51).In general, using laboratory markers such as white blood cell count, platelets ,and albumin. WBC count will elevate as part of the acute phase response (15). The platelets play an active role in many inflammatory conditions. (52) The increasing in platelet count correlates well with disease severity and may persist after bowel resection in IBD patients. Mean platelet volume has been hypothesized as a potential marker of clinical disease activity, The reduction in platelet volume in clinically active UC is still unknown, but its direct result of the thrombopoiesis disorder often observed in the early phases of systemic inflammatory progression (53).Moreover, the platelets also relates to the increased incidence of thromboembolic phenomena in CD and UC. Many studies showed that spontaneous platelet aggregation is found in more than 30% of IBD patients. (54)Furthermore , mean platelet count was increased in patients with active compared to inactive UC ($p = 0.008$) or healthy donors ($p = 0.000$).(55,56) ,but unlikely WBC ($10^9/L$) mean :7.99 range (7.16–8.8) p value : 0.123 within normal and Hb (g/L)) 123 (57,58) p value : 0.096 (20). Other laboratory markers, including WBC, platelets, and albumin have variable associations with the disease activity of IBD. In this study Platelets slightly increased but with no significance (59).Based on the present study and previous research, the IL-13 and PAF play critical roles in ulcerative colitis pathogenesis.

References:-

1. Baumgart DC, Sandborn WJ (2007). Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet 369:1641-1657.
2. Langholz E, Munkholm P, Davidsen M, Binder V. (1994). Course of ulcerative colitis: analysis of changes in disease activity over years. Gastroenterology 107: 3-11.
3. SatsangiJ,Silverberg MS, Vermeire S, Colombel JF(2006). The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 55(6):749-753
4. G. Bouma and W. Strober (2003). The immunological and genetic basis of inflammatory bowel disease. Nature ReviewsImmunology 3(7) :521–533.
5. Sierro S., Romero P., and Speiser D.E.(2011). The CD4-like molecule LAG-3, biology and therapeutic applications. Expert Opinionon Therapeutic Targets 15(1): 91–101.
6. Boirivant M, Fuss IJ, Chu A ,Strober W (1998). Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. J Exp Med 188 (10):1929–1939.
7. Rosen MJ, Frey MR, Washington MK, , Chaturvedi R, Kuhnhein LA, Matta P, Revetta FL, Wilson KT, Polk DB (2011). STAT6 activation in ulcerative colitis: a new target for prevention of IL-13-induced colon epithelial cell dysfunction. Inflamm Bowel Dis 17:2224–2234.
8. Larsen TB, Nielsen JN, Fredholm L, Lund ED, Brandslund I, Munkholm P, Hey H (2002). Platelets and anticoagulant capacity in patients with inflammatory bowel disease. Pathophysiol Haemost Thromb 32: 92-96.
9. Hocke M, Richter L, Bosseckert H, Eitner K (1999). Platelet activating factor in stool from patients with ulcerative colitis and Crohn's disease. *Hepato-gastroenterology* **46**(28):2333-2337.
10. Thyssen E., Turk J., Bohrer A., and StensonW.F (1996). Quantification of distinct molecular species of platelet activating factor in ulcerative colitis. *Lipids* 31 *Suppl.*: S255-S259.
11. Wardle T. D., Hall L., and Turnberg L.A.(1996) Platelet activating factor: release from colonic mucosa in patients with ulcerative colitis and its effect on colonic secretion. *Gut* **38**: 355-361.
12. Droy-Lefaix M. T., Drouet Y., Geraud G., Hosford D. and Braquet P.(1991). Superoxide dismutase (SOD) and the PAF-antagonist (BN 52021) reduce small intestinal damage induced by ischemia reperfusion. *Free Radic. Res. Commun*. (12–13): 725-735.

13. Pinsk V, Lemberg DA, Grewal K, Barker CC, Schreiber RA, Jacobson K (2007). Inflammatory bowel disease in the South Asian Pediatric Population of British Columbia *Am J Gastroenterology*. **102**:1077-1083.
14. . Plevy S (2004). Do serological markers and cytokines determine the indeterminate? *J Clin Gastroenterol* **38**: S51-S56
15. Langan R.C, Gotsch P.B, Krafczyk M.A, And SKILLINGE D.D (2007).Ulcerative colitis: Diagnosis and treatment. *American Family Physician* **76**, 1323-1330.
16. . *Ozin Y, Kilic MZY, Nadir I, Cakal B, Disibeyaz S, Arhan M, Dagli U, Tunc B, Ulker A, Sahin* B.(2009).Clinical Features of Ulcerative Colitis and Crohn's Disease in Turkey**.** J Gastrointestin Liver Dis. **18** (2):157-162.
17. Loftus EV, Jr., Shivashankar R, Tremaine WJ, Harmsen WS, Zinsmeiseter AR (2014).Updated Incidence and Prevalence of Crohn's Disease and Ulcerative Colitis in Olmsted County, Minnesota (1970-2011).ACG 2014 Annual Scientific Meeting.

18. Seong Won Han, MBBS, Elaine McColl,MSc John Roger Barton Peter James, Ian Nicholas Steen, and Mark Richard Welfare(2005). Predictors of Quality of Life in Ulcerative Colitis The Importance of Symptoms and Illness Representation, *Inflamm Bowel Dis* **11**:24-34.

19. Yeşil A , Şenateş E , Kayataş K , Koçhan K, Erdem ED , Şenateş BE , Gönen C (2013).Types and frequencies of extraintestinal involvement in our cases with inflammatory bowel disease. *Endoscopy Gastrointestinal***21**:15-18

20. Lees CW, Satsangi J.(2009). Genetics of inflammatory bowel disease: implications for disease pathogenesis and natural history. *Expert Rev Gastroenterol Hepatol*. **3**(5):513-534.
21. Shayesteh AA, Saberifirozi M, Abedian S, Sebghatolahi V (2013). Epidemiological, Demographic, and Colonic Extension of Ulcerative Colitis in Iran: A Systematic Review. *Middle East J Dig Dis* **5**:29-36.
22. Romanato G, Scarpa M, Angriman I, Faggian D, Ruffolo C, Marin R, Zambon S, Basato S, Zanoni S, Filosa T, Pilon F, Manzato E.(2009) Plasma lipids and inflammation in active inflammatory bowel diseases. Aliment Pharmacol Ther.**29** (3):298-307.
23. Ghazzawi I., AL-Mrayat Z (2007). Review of chronic ulcerative colitis cases at King Hussein Medical centre ,Jordan .*La Revue de Sant'e de la Meditranee' Oriental* **13**(2): 294-300.
24. Jain S, Pithadia A *(2008). Pathogenesis of inflammatory bowel disease (IBD)*. The Internet Journal of Gastroenterology 8 (1).

25. Fani A (2002). A study on the patteren of prevalence of inflamatory bowel disease and its clinical presentation in Arak. Rahavard Danesh Journal of Arak University of Medical Sciences 18(5):28-33.
26. . Shafeek MY, khafaj KR, Alkurri LE, fayadh MH, Al obaedy A (2006). Ulcerative colitis an endoscopic and histopathological comparison study .IJGE issue **6**(1):30-37.
27. Logan RF, Edmond ME, Somerville KW,Langman MS (1984) Smoking and Ulcerative Colitis. Br Med.J.(Clin.Res Ed),**288**(6419): 751-753
28. Harries AD, Baird A, Rhodes J. (1982).Non- smoking: a feature of ulcerative colitis. BMJ 284:706.
29. Sandler RS, Sandler DP, McDonnell CW, Wurzelmann JI (1992).Childhood exposure to environmental tobacco smoke and the risk of ulcerative colitis. *Am* J Epidemiol **135**:603-608

30. Miller LG, Goldstein G, Murphy M, Ginns LC (1982).Reversible alterations in immunoregulatory T cells in smoking. *Chest* **5**:527-529
31. Barton JR, Raid MA, Gaze MN, Maran AG, Ferguson A (1990). Mucosal immunodeficiency in smokers and in patients with epithelial head and neck tumours. Gut **31**:378-382

32. Bhatti MA, Hodgson HJF(1997). Peripheral blood proinflammatory cytokine in active inflammatory bowel disease: difference between smokers and non-smokers. Gut **40**:A24.
33. Calkins BM (1989). A meta-analysis of the role of smoking in inflammatory bowel disease. *Dig Dis Sci.***34**:1841-1854.
34. AL-Akashi R. , Subbar A (2000).Chronic Colitis, Clinical ,Endoscopic And Histological Evaluation In 130 Iraqi Patients. To F.I.C.M.S.GIT , Thesis

35. Boldeanu MV ,Silosi I ,Ghilusi M ,Cojocaru M ,Biciusca V ,Avramescu CS ,Cojocaru IM ,Ciurea T ,Albu DF ,Silosi CA (2014).Investigation of inflammatory activity in ulcerative colitis. *Rom J Morphol Emberyol* **55** (4) :1345-1351.
36. van J. Fuss, Frank Heller, Monica Boirivant, Francisco Leon, Masaru Yoshida, Stefan Fichtner-Feigl, Zhiqiong Yang, Mark Exley, Atsushi Kitani, Richard S. Blumberg, Peter Mannon and Warren Strober (2004). Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. *J Clin Invest* **113**(10): 1490–1497.
37. Boldeanu MV, Siloşi I, Ghiluşi M, Cojocaru M , Biciuşcă V, Avrămescu CS, Cojocaru MI, Ciurea T, Albu DF, Siloşi CA (2014). Investigation of inflammatory activity in ulcerative colitis. *Rom J Morphol Embryol* **55**(4):1345–1351.
38. Mannon P, Reinisch W (2012). Interleukin 13 and its role in gut defence and inflammation. *Gut* **61**(12):1765-1773.
39. Roda G, Marocchi M, Sartini A, and Roda E (2011). Cytokine Networks in Ulcerative Colitis. Hindawi Publishing Corporation. *Hindawi Publishing Corporation.***2011**:5.
40. Sventoraityte J, Zvirbliene A, Kiudelis G, Zalinkevicius R, Zvirbliene A, Praskevicius A, Kupcinskas L, Tamosiunas V (2008). Immune system alteration in patients with Inflamatory Bowel Disease during remission. *Medicina(Kaunas)***44**(1).
41. Bousvaros A, Antonioli DA, Colletti RB, Dubinsky MC, Glickman JN, Gold BD, Griffiths AM, Jevon GP, Higuchi LM, Hyams JS, Kirschner BS, Kugathasan S, Baldassano RN, Russo PA (2007).Differentiating ulcerative colitis from Crohn disease in children and young adults: report of a working group of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the Crohn's and Colitis Foundation of America. *J Pediatr Gastroenterol Nutr* **44**: 653-674.
42. Roberts-Thomson IC, Fon J, Uylaki W, Cummins AG, Barry S (2011). Cells, cytokines and inflammatory bowel disease: a clinical perspective. *Expert Rev* Gastroenterol Hepatol **5**: 703-716.
43. . Hocke M, Richter L, Bosseckert H, Eitner K (1999). Platelet activating factor in stool from patients with ulcerative colitis and Crohn's disease. Hepatogastroenterology **46**:2333–2337.
44. Caplan MS, HsuehW (1990). Necrotizing enterocolitis: role of platelet activating factor, endotoxin, and tumor necrosis factor. J Pediatr **117**(1): S47-S51.
45. Borthakur A, Bhattacharyya S, Alrefai WA, Joanne K. Tobacman, Ramaswamy K , and Dudeja PK (2010). Platelet-activating factor-induced NF-κ B activation and IL-8 production in intestinal epithelial cells are Bcl10 dependent. Inflamm Bowel Dis **16**(4):593-603.
46. Claud EC, Lu J, Wang XQ, Abe M, Petrof EO, Sun J, Nelson DJ, Marks J, Jilling T (2008). Platelet-activating factor-induced chloride channel activation is associated with intracellular acidosis and apoptosis of intestinal epithelial cells. Am J Physiol Gastrointest Liver Physiol. **294**:G1191–1200.
47. Sobhani I, Hochlaf S, Denizot Y, Vissuzaine C, Rene E, Benveniste J, Lewin MM, Mignon M.(1992). Raised concentrations of platelet activating factor in colonic mucosa of Crohn's disease patients. *Gut* **33**(9):1220-1225.
48. Appleyard CB, Hillier K (1995). Biosynthesis of platelet-activating factor in normal and inflamed human colon mucosa: evidence for the involvement of the pathway of platelet-activating factor synthesis de novo in inflammatory bowel disease.Clin Sci (Lond). **88(**6):713-717.
49. Ford AC, Khan KJ , Sandborn WJ , Kane SV ,Moayyedi P (2011). Once -Daily Dosing vs. Conventional Dosing Schedule of Mesalamine and Relapse of Quiescent Ulcerative Colitis: Systematic Review and Meta-Analysis. Am J Gastroenterol **106**(12):2070-2077.
50. D'Haens G, Sandborn WJ, Barrett K, Hodgson I, Streck P (2012). Once –Daily MMX Mesalamine for Remission of Ulcerative Colitis. Am J Gastroenterol. **107**(**7**):1064-1077.
51. Yilmas B, Koklu S, Yuksel O, Arslan S (2014).Serum beta 2- microglobulin as a biomarker in inflammatory bowel disease. World Journal of Gastroenterology. **20**(31): 10916–10920.
52. Danese S, Motte Cd Cde L, Fiocchi C (2004). Platelets in inflammatory bowel disease: clinical, pathogenic, and therapeutic implications. *Am J Gastroenterol* 99: 938-945
53. Kapsoritakis AN, Koukourakis MI, Sfiridaki A, Potamianos SP, Kosmadaki MG, Koutroubakis IE, Kouroumalis EA (2001). Mean platelet volume: a useful marker of inflammatory bowel disease activity. *Am J Gastroenterol* **96**: 776-781.
54. Webberley MJ, Hart MT, Melikian V (1993). Thromboembolism in inflammatory bowel disease: role of platelets. *Gut* **34**: 247-251.

55. Hasan Kayahan, Mesut Akarsu, Mehmet Ali Ozcan, Serdal Demir, Halil Ates, Belkis Unsal, Hale Akpinar (2007). Reticulated platelet levels in patients with ulcerative colitis. International Journal of Colorectal Disease.**22** (12):1429-1435.

56. Han SW, McColl E, Barton JR, James P, Steen IN, Welfare MR.(2005). Predictors of quality of life in ulcerative colitis: the importance of symptoms and illness representations. Inflamm Bowel Dis. **11**(1):24-34.
57. Humphrey DM, McManus LM, Satouchi K, Hanahan DJ, Pinckard RB(1982). Vasoactive properties of acetyl glyceryl ether phosphorycholine and analogues. *Lab Invest* **46**: 422-7**.**
58. McManus LM, Hanahan DJ, Demopoulos CA, Pinckard RN (1980). Pathobiology of the intravenous infusion of acetyl glycerol ether phosphorycholine (AGEPG), a synthetic platelet activating factor (PAF) in the rabbit. J Immunol **124**: 2919-2924.
59. Naidoo CMY, Leach ST, Day AS, Lemberg DA (2014). Inflammatory Bowel Disease in children of Middle Eastern Decent. *International Journal of Pediatrics*., Article ID 906128, P7.