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The Assessment of Calprotectin Protein as a Marker in the Ulcerative Colitis and Colorectal Cancer Patients

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ABSTRACT

The aim of study is Assessment of plasma CP as a marker compared to fecal CP in patients with UC and CRC. Sixty patients (45) were UC and (15) were CRC, during the period from 1st of December 2013 till 30th of May 2014. The practical side was performed at the laboratory of Biochemistry Department, College of Medicine, University of Babylon. In this study all cases were proved by histopathological confirmation. The plasma obtained from the blood of patients were used to measure the concentrations of plasma and fecal calprotectin (CP), by using Enzyme Linked Immune Sorbent Assay (ELISA), the serum separated from the blood control group were tested by CRP testing to exclude the inflammatory conditions extracted stool was used to measure the concentration of fecal CP for patients, and gel electrophoresis done on extracted stool of five samples from UC and CRC with their controls

Keywords: Ulcerative Colitis, Colorectal Cancer, plasma Calprotectin, fecal Calprotectin.

INTRODUCTION

Ulcerative colitis(UC) is a chronic disease of the large intestine, also known as the colon, in which the lining of the colon becomes inflamed and develops tiny open sores, or ulcers, that produce puss and mucous. The combination of inflammation and ulceration may cause abdominal pain, discomfort, frequent emptying of the colon and in the acute cases bloody diarrhea.

Colorectal cancer (CRC) is cancer that starts in the colon or the rectum. These cancers can also be referred to separately as colon cancer or rectal cancer, depending on where they start. Colon cancer and rectal cancer have many features in common.

Ulcerative colitis (UC) is a chronic inflammatory condition characterized by relapsing and remitting episodes of inflammation limited to the **mucosal layer of the colon**. It almost invariably involves the rectum and typically extends in a proximal and continuous fashion to involve other portions of the colon. Different terms have been used to describe the degree of involvement [1]. Colorectal cancer (CRC) is the second leading cause of cancer death worldwide. About 1 million CRC cases occur each year, of which 500000 people will die [2]. Survival rates are closely related to the stage of cancer at the time of diagnosis

and the most promising approach to reducing mortality rates is early detection of precancerous or cancerous lesions [3].

Calprotectin,(CP) also known as MRP-8/MRP-14 or S100A8/A9 heterocomplex, is formed out of the calcium-binding, migration inhibitory factor-related proteins, MRP-8 (S100A8) and MRP-14 (S100A9.The expression of these proteins is largely confined to the cytosol of neutrophils and monocytes[4].The complex formation of CP is calcium-dependent. CP comprises 60% of the cytoplasmic protein fraction of circulating polymorphonuclear granulocytes and is also found in monocytes, macrophages and ileal tissue eosinophils. Peripheral blood monocytes carry the antigen extra- and intracellularly, neutrophils only intracellularly[5]. CP has antibacterial, antifungal, immunomodulating and ant proliferative effects. Furthermore, it is a potent chemotactic factor for neutrophils. Plasma concentrations are elevated in diseases associated with increased neutrophil activity. During intestinal wall inflammation, granulocytes transmigrate through the intestinal wall. Therefore CP is also detectable in fasces [6,7]. Molecular structure of CP and its subunits S100A8, also known as cystic fibrosis (CF) antigen, MRP8, L1 light chain, p8, calgranulin A and S100A9, also known as MRP14, L1 heavy chain, p14, calgranulin B, Murine S100A8, originally called chemotactin protein 10 (CP10), has 59% amino acid sequence identity with human S100A8 and 64% nucleotide identity [8]. CP is an interesting peptide, proposed as a biomarker for various inflammatory diseases due to its potential role in pathophysiology of inflammation associated outcomes like tissue destruction, apoptosis and growth impairment. As an acute phase reactant, calprotectin increases more than 100 folds during inflamed conditions [9].

The aim of study is assessment of plasma CP as a marker compared to fecal CP in patients with UC, CRC determine the specificity, sensitivity, predicted positive and negative values of CP, evaluation of optimal cutoff value anddetection probability of abnormal protein bands by electrophoresis of extracted stool samples in UC and CRC.

MATERIALS AND METHODS

All cases were proved by histopathologicalconfirmation.The plasma obtained from the blood of patients were used to measure the concentrations of plasma calprotectin (CP), by using Enzyme Linked Immunoe Sorbent Assay (ELISA)**.**

This study was performed at the laboratory of Biochemistry Department, College of Medicine, University of Babylon. The collection of samples was conducted during the period from 1st of December 2013 till 30th of May 2014**.**The group of patients are subjected in this study were (60) individuals with overall mean age of patients was (48.08± 15.32) years old and majority (83.3%) of patients aged less than 60 years. The majority (71.7%) of patients were males.

All of those patients are admitted to Babylon gastrointestinology center in Hila city with clinical symptoms of inflammatory bowel disease. The diagnosis and the type of ulcerative colitis and colorectal cancer were confirmed by endoscopy technique and histological testing. Only patients with UC and CRC were included in this study.

1. All patients were presented in the acute state of disease of UC and various stages of CRC.
2. The patients whose clinical symptoms and imaging confirming no UC and CRC, were excluded from this study.
3. The verbal consent was taken from the patient directly or from his or her escort to be contributed in this study.

All cases were proved by histopathologicalconfirmation.The plasma obtained from the blood of patients were used to measure the concentrations of plasma calprotectin (CP), by using Enzyme Linked Immunoe Sorbent Assay (ELISA), the serum separated from the blood control group were tested by CRP testing to

exclude the inflammatory conditions extracted stool was used to measure the concentration of fecal CP for patients, and gel electrophoresis done on extracted stool of five samples from UC and CRC with their controls .

Statistical Analysis: All statistical analysis was performed by using SPSS 17 version. Case control study design is used to prove the significant differences in plasma CP with UC and CRC. Data were expressed as (mean ± SD). The normality of the distribution of all variables was assessed by the student′s t-test and Pearson correlation analyses that have been used to determine the significant difference between the groups. Specificity, sensitivity, negative and positive predicted values are performed by using ROC carve. P values less than (0.05) is considered significant and less than (0.01) is considered highly significant.

RESULTS AND DISCUSSION

The results showed significant association between plasma calprotectin and control group mine while showed the same association with fecal calprotictin in both two disease UC and CRC. The Results found are presented in tables 1-15.

Table 1: Shows Mean difference of plasma calprotectin by patients and control groups

Variable	Study Groups	N	Mean	Standard Error	t-test	P value
Plasma CP	Patients Control	60 60	4367.67 1003.63	600.58 118.98	**5.495**	**<0.001***

*P value ≤0.05 is significant

Table 2: Shows Mean difference of fecal and plasma CP by patients' type of bowel disease

Variable	Types of bowel disease	N	Mean± SD	Standard Error	t-test	P value
Fecal calprotectin	Ulcerative colitis Colo-Rectal cancer	45 15	6056.87± 2122.83 24484.93± 20488.97	316.45 5290.23	**6.039**	**<0.001***
plasma calprotectin	Ulcerative colitis Colo-Rectal cancer	45 15	3043.02± 1186.52 8341.60± 8013.75	176.52 8013.75	**4.366**	**<0.001***

*P value ≤0.05 is significant

Table 3 : Shows Association between types of bowel disease and each of age and sex

Variable	Types of bowel disease		X^2	P value
	Ulcerative colitis (%)	Colo-Rectal cancer (%)		
Age group ≤ 60 years > 60 years	44 (97.8) 1 (2.2)	6 (40.0) 9 (60.0)	**27.040**	**<0.001***
Sex Male Female	32 (71.1) 13 (28.9)	11 (73.3) 4 (26.7)	0.027	0.869

*P value ≤0.05 is significant

Table 4: Association between fecal and plasma calprotectin for detection of ulcerative colitis at 3000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 3000 ng/ml (%)	>3000 ng/ml (%)	Total	
plasma Calprotectin				
≤3000 ng/ml	5 (100.0)	15 (37.5)	20 (44.4)	
>3000 ng/ml	0 (0.0)	25 (62.5)	25 (55.6)	**0.013***
Total	5 (100.0)	40 (100.0)	45 (100.0)	

*P value ≤0.05 is significant

Table 5: Area under the curve for detecting ulcerative colitis by plasma calprotectin comparing by fecal calprotectin at 3000 cutoff point.

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.188	0.070	0.024*	0.051	0.324

Table 6: Association between fecal and plasma calprotectin for detection of colo-rectal cancer at 3000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 3000 ng/ml (%)	>3000 ng/ml (%)	Total	
plasma Calprotectin				
≤3000 ng/ml	6 (100.0)	2 (22.2)	8 (53.3)	
>3000 ng/ml	0 (0.0)	7 (77.8)	7 (46.7)	**0.007***
Total	6 (100.0)	9 (100.0)	15 (100.0)	

P* value ≤ 0.05 is significant

Table 7: Area under the curve for detecting colo-rectal cancer by plasma calprotectin comparing by fecal calprotectin at 3000 cutoff point

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.111	0.090	0.013*	<0.001	1.000

Table 8: Association between fecal and plasma calprotectin for detection of ulcerative colitis at 2000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 2000 ng/ml (%)	>2000 ng/ml (%)	Total	
Plasma Calprotectin				
≤2000 ng/ml	4 (100.0)	3 (7.3)	7 (15.6)	
>2000 ng/ml	0 (0.0)	38 (92.7)	38 (84.4)	**<0.001***
Total	4 (100.0)	41 (100.0)	45 (100.0)	

*P value ≤0.05 is significant

Table 9: Area under the curve for detecting ulcerative colitis by plasma calprotectin comparing by fecal calprotectin at 2000 cutoff point

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.963	0.027	0.002*	0.000	1.000

Table 10: Association between fecal and plasma calprotectin for detection of colo-rectal cancer at 2000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 2000 ng/ml (%)	>2000 ng/ml (%)	Total	
plasma Calprotectin				
≤2000 ng/ml	5 (100.0)	1 (10.0)	6 (40.0)	**0.001***
>2000 ng/ml	0 (0.0)	9 (90.0)	9 (60.0)	
Total	5 (100.0)	9 (100.0)	15 (100.0)	

*p value ≤ 0.05 is significant

Table 11: Area under the curve for detecting colo-rectal cancer by plasma CP comparing by FC at 2000 cutoff point

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.950	0.060	0.006*	0.001	1.000

Table 12: Association between fecal and plasma calprotectin for detection of ulcerative colitis at 1000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 1000 ng/ml (%)	>1000 ng/ml (%)	Total	
plasma Calprotectin				
≤1000 ng/ml	1 (100.0)	3 (6.8)	4 (8.9)	0.089[a]
>1000 ng/ml	0 (0.0)	41 (93.2)	41 (91.1)	
Total	1 (100.0)	44 (100.0)	45 (100.0)	

*p value ≤ 0.05 is significant ,[a:] Fisher Exact test

Table 13: Area under the curve for detecting ulcerative colitis by plasma calprotectin comparing by fecal calprotectin at 1000 cutoff point

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.375	0.170	0.0414	0.041	0.709

Table 14: Association between fecal and plasma calprotectin for detection of colo-rectal cancer at 1000 cutoff point

Variable	FecalCalprotectin			*P* values
	≤ 1000 ng/ml (%)	>1000 ng/ml (%)	Total	
plasma Calprotectin				
≤1000 ng/ml	1 (100.0)	3 (21.4)	4 (26.7)	
>1000 ng/ml	0 (0.0)	11 (78.6)	11 (73.3)	**0.367**[a]
Total	1 (100.0)	14 (100.0)	15 (100.0)	

[a:] Fisher Exact test

Table 15: Area under the curve for detecting colo-rectal cancer by plasma calprotectin comparing by fecal calprotectin at 1000 cutoff point

Area	Std. Error	P value	95% CI	
			Lower Bound	Upper Bound
0.375	0.183	0.437	0.017	.733

Ulcerative Colitis (UC) is a chronic idiopathic condition, marked by recurrent episodes of inflammation of the GIT, interspersed with periods of remission[10]. Colorectal cancer is associated with a local acute inflammatory reaction so that in some cases it can be visualized by white cell neutrophil scanning [11]. CRC is the third commonly cancer diagnosed cancer in both men and women. This study agree with the previous studies Sugita et al mean age (49 ±) which confirm that UC and CRC diseases associated with age [12] but do not with sex.

Various indexes are used to evaluate the activity of UC, which differ from each other in terms of being more subjective (clinical), more objective (endoscopic-histological) or a combination of the two. However, despite the different indexes available, there is not yet any consensus in the literature as to which is the most valid [13]. Laboratory parameters such as C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and hemoglobin, among others, are not specific to active UC, which makes it difficult to use them routinely as markers of inflammatory activity in clinical practice. Indeed, these markers correlate poorly with endoscopic and histological examination [14].

Fecal CP is a stable neutrophil specific marker which can be assayed in stool with high precision and ease. Within the neutrophil calprotectin is found in the extra lysosomal cytosol and constitutes up to 60% of the total protein content [15]. Levels of fecal CP are increased in patients with colorectal cancer but immunohistochemical examination of colorectal cancer specimens has shown reactivity confined to neutrophilic granulocytes with no reactivity seen in neoplastic cells, suggesting that elevated fecal levels may be due to neutrophil. The presence of CP in feces is directly proportional to neutrophil migration toward the intestinal tract [15].

The present study was to assess fecal and plasma CP as markers of UC and CRC and how these markers associated with each other at different cutoff values. Furthermore sensitivity, specificity, positive predicted value and negative predicted value were calculated also. It can be found the mean difference of fecal and plasma CP between UC and CRC is significant. The high levels associated with colorectal carcinoma are likely caused by:

1. Polymorphonuclear cell infiltration of the tumor and subsequent shedding into the intestinal lumen.

2. The recruitment of such cells by the tumor is almost certainly a consequence of the local production of chemotactic factors, possibly in response to a breach in the protective mucosal lining.[16]

Several studies have compared fecal calprotectin with activity indexes and/or endo scopic / histological evaluation to verify intestinal inflammation in IBD patients. The results of these studies are promising, having demonstrated that this marker is useful in detecting inflammation [17]. Some Authors consider a colonoscopy with biopsy to be the best means for evaluating inflammation location, extent, and severity; aside from being an invasive method, this approach carries risks of complications [18]. Various studies have described fecal CP as powerful biomarker of inflammation of the intestinal mucosa in patients with UC and CRC. Fecal CP selected and studied as indicator of inflammation [19].

Many authors have claimed that CP levels correlate closely with histological evaluation than macroscopic findings, suggesting that this biological marker is more sensible than endoscopy in evaluating IBDs activity [20]. Furthermore fecal CP concentrations predicted the severity of colorectal inflammation, with advance d histological grades of colorectal inflammation. In previous study Haer etal was suggested that the fecal CP is a good marker for detect UC at cutoff value (400 µg g^{-1}) the sensitivity of fecal CP is (68%) and specificity is (69%) [21].This study is the first to find significant association between plasma and fecal CP at two different cutoff values (2000,3000)ng mL^{-1} , the sensitivity and negative predicted values of plasma CP at these cutoffs were (100%) however, the specificity were (92.7%, 62.5%) in UC and (90%, 77.8%) in CRC, meanwhile the positive predicted values were (57.1%, 83.3%) in UC and (25%,75%) in CRC respectively . Plasma CP was succeed to detect the UC and CRC only at the cutoff value (2000) ng mL^{-1} and was highly sensitive and more specific, however these cutoff value can be consider the optimal cutoff value to detect these two disease and possible to be used as a diagnostic marker.

It was observed that there was no significant association between plasma and fecal CP at (1000) ng/ml cutoff value, the sensitivity and negative predicted values of this marker at this cutoff were (100%). However, the specificities were (93.2% in UC and (75%) in CRC, meanwhile the Plasma CP was failed to detect the UC and CRC at this cutoff in spite of the high sensitivity and specificity Plasma CP at 1000 ng mL^{-1} is useful in screening for these two disease.

In this study it was tried to migrate the proteins present in the exacted stool by electrophoresis in an attempt to find an abnormal band. There was no any abnormal additional band appeared from this migration in UC and CRC patients, the only observed abnormality was an increase in gamma region compared to control group[22].

APPLICATIONS

This study is useful for further studies about Patients.

CONCLUSIONS

The plasma CP in UC and CRC is significantly associated with fecal CP at two different cut off values (2000, 3000)ng mL^{-1}, while it is not significantly associated with faecal CP at cut-off value (1000)ng mL^{-1}.The optimal cut off value is 2000 ng mL^{-1}, the plasma CP success to detect at these cut off.

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