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Pathology

ROLE OF FECAL CALPROTECTIN FOR ASSESSMENT OF INFLAMMATORY BOWEL DISEASE ACTIVITY, 4-YEAR STUDY DONE IN INDIAN REFERENCE LABORATORY

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ABSTRACT **Introduction:-**Calprotectin is calcium binding protein which is secreted by neutrophils & monocytes. It is found both in plasma & in stools. It is predominantly elevated in inflammatory conditions such as inflammatory bowel disease (IBD). To a certain extent, it is also elevated in infectious conditions & in polyposis. This study was conducted to evaluate diagnostic precision of fecal calprotectin in known inflammatory bowel disease (IBD) patients **Method:-** A retrospective analysis of calprotectin, ASCA, cANCA, pANCA, Total WBC count, ESR, CRP was obtained in referral laboratory in Mumbai over a span of 4 years (2018-2022). **Results:-** Prevalence of raised calprotectin was significantly seen in 13-18 years age group. (P<0.0001). Calprotectin showed a positive correlation with WBC count, CRP & ESR. There was no significant association between calprotectin levels & gender. **Conclusion:-** Fecal calprotectin can be used not only in research but also in routine clinical practice in differentiating between patients with inflammatory & non-inflammatory bowel disease.

Aims and Objectives: -

1. To study the prevalence of fecal calprotectin levels with respect to different age groups & gender in inflammatory bowel disease patients.
2. To establish correlation of fecal calprotectin levels with ANCA, ASCA, Total WBC count, ESR &CRP

KEYWORDS : IBD, Calprotectin, ASCA, ANCA

INTRODUCTION

Incidences of inflammatory bowel disease (IBD) cases is increasing worldwide. Ulcerative colitis & Chron's disease are major sub types of inflammatory bowel disease. Clinically they have episodes of flare-ups followed by remissions[1-4]. Conventional markers such as C- reactive protein, erythrocyte sedimentation rate, & total leukocyte count, shows systemic host response only, as they are not specific for intestinal inflammation [5]. Endoscopy is currently considered as gold slandered for evaluation of mucosal inflammation[6].However, being an invasive, time consuming, expensive process, it may not be preferred by every patient. Hence, fecal calprotectin is preferred by clinicians to assess flare-ups for timely intensification of treatment and better disease control. Fecal calprotectin was first described by Fagerhol et al in 1980[7] and was known as L1 protein, MRP-8/14, calgranulin & cystic fibrosis antigen [8-11]. It weighs 36 Kilo-Dalton and has anti-microbial & anti-proliferative properties. Fecal calprotectin constitutes roughly 60% of cytosolic protein in neutrophils [12]. It is excreted in feces & can be measured with ELISA/CLIA[13]. It resists the degradation caused by intestinal bacteria & can remain stable at room temperature up to one week [14] Inflammatory & neoplastic conditions such as ulcerative colitis, Crohn's disease & colorectal carcinoma cause elevation in calprotectin levels[15,16]. In ulcerative colitis patients, calprotectin level correlates with the histological & endoscopic assessment findings [17]. In patients suffering from Crohn's disease calprotectin value correlates with radio-labelled white cell scanning which is used for assessing disease activity [17]. In addition to this, calprotectin also helps in predicting relapse of inflammatory bowel disease.[18]

MATERIALS & METHODS

This retrospective study was conducted over a period of 4 years from 2018-2022. A total 38,660 test results were included in this study from Mumbai City. The stool samples were analysed for calprotectin levels, and the serum samples were tested for Total WBC count, Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Anti-nuclear cytoplasmic antibody (p-ANCA & c-ANCA) and Anti-Saccharomyces cerevisiae antibody (ASCA).

Calprotectin levels were measured on Liason XL (CLIA), ASCA levels were analysed on Alegria (ELISA), Analysis of c-ANCA & p-ANCA levels were measured on Sprinter XL(ELISA), Total WBC count was analysed on DXH coulter (5&7 parts), ESR was measured using cube 30 and CRP was measured using Cobas analyser.

Biological reference range

Biological reference ranges of above mentioned parameters were as follows:

Parameter	Biological reference range	Reference
Total WBC count	4,300-10,300/mm3	Dacie & Lewis practical hematology book, 12th edition, 2016
Erythrocyte sedimentation rate (ESR)	0-15 mm/hr	Dacie & Lewis practical hematology book, 12th edition, 2016
Calprotectin	<50 µg/g	1. Faecal calprotectin diagnostic tests for inflammatory diseases of the bowel. NICE diagnostics guidance [DG11]: October 2013 2. Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. Costa, Ceccarelli, et al. Gut 2005 54: 364-368

Data recording was done and the discrete variables are summarized in terms of frequencies and percentages. Pearson's correlation coefficient was used to analyse relation between calprotectin level with total WBC count, CRP, ASCA IgG and ESR and for comparison of categorical variable Chi Square test was used.

All statistical analysis was performed using “R Studio version 1.4.1103”. A two-tailed p value of <0.05 was considered to be statistically significant.

RESULTS

Total 38,660 cases were studied over period of four years from 2018-

2022 of which majority cases belonged to age group 19-45 Years (49.63%). The percentage of males were more as compared to females (54.15% vs 45.85%).

Table 1: -Age and Gender wise distribution

	Frequency	Percentage
Age Group(In years)		
1 - 12	3082	8.04
13 - 18	2023	5.28
19 - 45	19020	49.63
46 - 60	7889	20.58
>60	6311	16.47
Gender		
Female	17725	45.85
Male	20935	54.15

(Table:-1)

Out of the total cases, calprotectin levels were elevated in 12,102 patients (31.30%). Of the total samples, 704 cases had done ASCA IgA, 645 cases had done ASCA IgG of which 10.23% cases had positive IgA, and 27.41% had Positive IgG.

C-ANCA and P-ANCA were done in 376 cases, only 0.80% cases were positive for C-ANCA and 1.86% cases were positive for p-ANCA.

Total 208 (10.08%) patients had high WBC count & 119 (5.77%) had low WBC count. CRP were raised in 525 (30.92%) patients

Table 2: - Frequency Distributions of diagnostic parameters

	Frequency	Percentage
Calprotectin		
Abnormal	12102	31.30
Normal	26558	68.70
ASCA IgA		
Negative	632	89.77
Positive	72	10.23
ASCA IgG		
Equivocal	4	0.62
Negative	462	71.96
Positive	176	27.41
c- ANCA		
Negative	373	99.20
Positive	3	0.80
p-ANCA		
Negative	369	98.14
Positive	7	1.86
Total Leucocytes (WBC)		
High	208	10.08
Low	119	5.77
Normal	1736	84.15
CRP		
Abnormal	525	30.92
Normal	1173	69.08

(Table:-2)

A Statistical significant association of age group was observed with Calprotectin (p value <0.0001), with maximum abnormality seen in age group of 13 – 18 years (38.06%) followed by 1 – 12 years (35.79%). Gender did not have any association with Calprotectin (p value = 0.6372).

Table 3: - Comparison of calprotectin levels across different age groups and gender

	Calprotectin				
		Abnormal	Normal		p value
	N	%	N	%	
Age Group					
1 - 12	1103	35.79	1979	64.21	<0.0001
13 - 18	770	38.06	1253	61.94	
19 - 45	5649	29.70	13371	70.30	
46 - 60	2345	29.72	5544	70.28	
>60	2162	34.26	4149	65.74	
Gender					
Female	5570	31.42	12155	68.58	0.6372
Male	6532	31.20	14403	68.80	

(Table:-3)

Calprotectin was compared with different parameters such as c-ANCA, p-ANCA, total leucocyte count, ASC IgA &IgG, CRP & ESR. Calprotectin levels have statistically significant association (P<0.001) with WBC count, CRP & ESR.

Table: - 4 Comparison of calprotectin levels with different parameters

	Calprotectin				
	Abnormal			Normal	p value
	N	%	N	%	
C ANCA					
Negative	153	41.02	220	58.98	-
Positive	3	100.00	0	0.00	
P ANCA					
Negative	152	41.19	217	58.81	0.3968
Positive	4	57.14	3	42.86	
Total Leucocytes (WBC)					
High	111	53.37	97	46.63	<0.0001
Low	33	27.73	86	72.27	
Normal	555	31.97	1181	68.03	
ASCA IgA					
Negative	242	38.29	390	61.71	0.7183
Positive	26	36.11	46	63.89	
ASCA IgG					
Equivocal	2	50.00	2	50.00	0.7796
Negative	178	38.53	284	61.47	
Positive	72	40.91	104	59.09	
CRP					
Abnormal	266	50.67	259	49.33	<0.0001
Normal	300	25.58	873	74.42	
ESR					
Abnormal	222	41.65	311	58.35	<0.0001
Normal	168	23.76	539	76.24	

(Table:-4)

Weak positive correlation was observed for total leucocytes (r=0.1810, p value=<0.0001), CRP (r=0.2012, p value=<0.0001) and ESR (r=0.2163, p value=<0.0001) with Calprotectin.

Table: - 5: Correlation of calprotectin with different parameters

Parameter	Correlation Coefficient (r)	95% of r	p value
Total Leucocytes	0.1810	0.1389 to 0.2224	<0.0001
CRP	0.2012	0.1551 to 0.2464	<0.0001
ASCA IgA	-0.01136	-0.08561 to 0.06301	0.7648
ASCA IgG	0.08414	0.006193 to 0.1611	0.0344
ESR	0.2163	0.1625 to 0.2687	<0.0001

(Table:-5)

Statistically significant findings of the study: -

1. Raised calprotectin levels were seen in 13-18 years' age group.
2. Calprotectin levels had positive correlation with WBC count, CRP & ESR levels

DISCUSSION

Chronic relapsing and remitting inflammation of the gastrointestinal tract is the hallmark of inflammatory bowel disease. One of the most prominent histological features observed in ulcerative colitis is infiltration of the neutrophils into the inflamed mucosa at an early stage of inflammation. The neutrophils are major sources of inflammatory cytokines, chemokines, proteases, active lipids, and reactive oxygen derivatives. They also activate complement system, which exacerbates mucosal inflammation and tissue injury. [19-21] The fecal calprotectin excretion of indium-labeled autologous granulocytes was considered as gold standard test in assessing bowel inflammation in inflammatory bowel disease.[22] However, as it involves an exposure to radiation and prolonged fecal collection, it was very much uncomfortable for patients as well as for laboratory staffs. Hence, it is only used as a research tool.

In our study, we focused on the evaluation of any relationship that might exist between the mucosal neutrophil infiltration which is

represented by calprotectin, CRP, raised WBC count & ESR. We found out that, raised calprotectin levels, has got significant correlation with raised WBC count, raised ESR & raised CRP levels. These findings were consistent with study done by Tibble et al. [23] Costa et al also reported similar findings, and proved efficacy of fecal calprotectin diagnosing relapse of ulcerative colitis[24]

In our study, CLIA was used to determine the fecal calprotectin concentrations in the patients. In comparison to endoscopy, this method is simple, noninvasive and inexpensive. However, fecal calprotectin can only reflect the excretion of neutrophils. Many infective diseases can cause a large number of neutrophils to infiltrate, so that fecal calprotectin is elevated in a number of organic gastroenterological disorders [25-27] Therefore, fecal calprotectin is not desirable as a method that is required to differentiate efficiently between inflammatory bowel disease and infective colitis. Therefore, it cannot replace endoscopy in diagnosing inflammatory bowel disease. Regardless of how sensitive the calprotectin technique may be in the detection of disease activity in patients with previously diagnosed UC, its greater potential use is in identifying and differentiating between patients with inflammatory bowel disease & non-inflammatory bowel disease.

CONCLUSION

The determination of fecal calprotectin is an objective approach to grading the mucosal disease activity in patients with inflammatory bowel disease. The advantages of fecal calprotectin are simplicity, noninvasiveness, and relatively low cost. Hence it can be said that, fecal calprotectin can be useful not only in research but also in routine clinical practice

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