



## Investigating the Influence of Fecal Calprotectin Levels on the Progression of Crohn's Disease in Smokers and Non-Smokers

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### Abstract

Crohn's disease, a prevalent gastrointestinal disorder, exhibits significant variations in disease severity and outcomes among individuals. This study seeks to investigate the fecal calprotectin concentration in Crohn's disease patients, comparing those who smoke and those who do not. Stool samples were meticulously collected in sterile containers from both smoking and non-smoking Crohn's disease patients to detect calprotectin levels using the CHORUS apparatus. The established threshold values for interpretation were as follows: A POSITIVE result indicated calprotectin levels greater than 60 mg/kg, while a NEGATIVE result indicated levels less than 40 mg/kg. The current study enrolled a total of 60 Crohn's disease patients, with 30 identified as smokers and the remaining 30 as non-smokers. Our findings revealed that the concentration of fecal calprotectin in Crohn's disease patients was notably elevated in those who were tobacco users compared to their non-smoking counterparts. Moreover, a distinct pattern emerged when examining the age groups of the participants. Specifically, the mean  $\pm$  standard deviation (SD) level of stool calprotectin was observed to be elevated in the age group of 30-49 years among the smoking patients, whereas lower results were recorded in the age group of 10-29 years among non-smoking patients. These results underscore the potential impact of smoking on fecal calprotectin levels in Crohn's disease patients and the role that age may play in this association. Further research and clinical investigation are warranted to explore the mechanisms behind these observed differences and their clinical implications.

**Keyword:** Crohn's disease, Stool Calprotectin, smoking and non-smokin.

### التحقيق في تأثير مستويات كالبروتكتين البراز على تطور مرض كرون لدى المدخنين وغير المدخنين

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### الخلاصة

يظهر مرض كرون، وهو اضطراب معدي معوي سائد، اختلافات كبيرة في شدة المرض ونتائجه بين الأفراد. تسعى هذه الدراسة إلى التحقيق في تركيز كالبروتكتين البراز لدى مرضى مرض كرون، ومقارنة أولئك الذين يدخنون وأولئك الذين لا يدخنون. تم جمع عينات البراز بدقة في حاويات معقمة من كل من مرضى مرض كرون المدخنين وغير المدخنين للكشف عن مستويات الكالبروتكتين باستخدام جهاز CHORUS. وكانت القيم الحدية

المحددة للتفسير كما يلي: أشارت النتيجة الإيجابية إلى مستويات كالبروتيكيتين أكبر من 60 مغ/كغ، في حين أشارت النتيجة السلبية إلى مستويات أقل من 40 مغ/كغ. سجلت الدراسة الحالية ما مجموعه 60 مريضاً بمرض كرون، مع تحديد 30 على أنهم مدخنون والباقي 30 على أنهم غير مدخنين. كشفت النتائج التي توصلنا إليها أن تركيز كالبروتيكيتين البراز في مرضى مرض كرون كان مرتفعاً بشكل ملحوظ لدى أولئك الذين كانوا من مستخدمي التبغ مقارنة بنظرانهم غير المدخنين. علاوة على ذلك، ظهر نمط مميز عند فحص الفئات العمرية للمشاركين. على وجه التحديد، لوحظ ارتفاع متوسط مستوى الانحراف المعياري  $\pm$  (SD) كالبروتيكيتين البراز في الفئة العمرية 30-49 سنة بين المرضى المدخنين، في حين تم تسجيل نتائج أقل في الفئة العمرية 10-29 سنة بين المرضى غير المدخنين. تؤكد هذه النتائج على التأثير المحتمل للتدخين على مستويات كالبروتيكيتين البرازية لدى مرضى مرض كرون والدور الذي قد يلعبه العمر في هذا الارتباط. هناك ما يبرر إجراء مزيد من البحوث والتحقق السريرية لاستكشاف الآليات الكامنة وراء هذه الاختلافات الملحوظة وآثارها السريرية.

## 1. Introduction

Crohn's disease is a chronic inflammatory bowel disorder characterized by recurrent episodes of intestinal inflammation that can result in progressive bowel damage and disability (1). These relapsing inflammatory episodes are the hallmark of Crohn's disease, often leading to irreversible gastrointestinal damage (2). This debilitating condition can affect individuals of all ages (3,4) and significantly impacts their quality of life. Various studies have highlighted the role of environmental factors as crucial contributors to Crohn's disease development, although initial findings were inconclusive (5-11). Genetic susceptibility remains a primary factor in the onset of Crohn's disease, with interactions between genetic and environmental factors triggering alterations in the intestinal microbiome and subsequently influencing host immunity (12). As the disease progresses, environmental factors become increasingly influential compared to genetic influences, with genetic factors predominantly affecting early-onset Crohn's disease. In contrast, the impact of environmental factors tends to become more pronounced in the later stages of the disease (13).

Calprotectin, a protein primarily produced in neutrophil granules (14), has gained recognition as a valuable screening tool to differentiate between irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), a role supported by recent research (15). Furthermore, fecal calprotectin (FC) serves as a marker to assess the prognosis of Crohn's disease status (16-18). It is commonly employed in clinical practice as a non-invasive biomarker that strongly correlates with disease activity and guides therapeutic decisions (19,20). FC may also offer utility as a marker to predict the development and progression of Crohn's disease. This potential is explored by examining evidence linking changes in FC levels to specific exposures and their associated risks of Crohn's disease development. Numerous studies have reported a correlation between FC levels and endoscopic measures of disease activity (21-25). The primary objective of this study is to investigate the role of fecal Calprotectin in the progression of Crohn's disease among both smoking and non-smoking patients.

## 2. Materials and Methods

The data for this case study was gathered at the Medicine Department of the General Nasiriya Hospital in Iraq, Thi-Qar governorate, during the period spanning from May to August 2023. Patients of all ages and sexes were included in the study, while those who had undergone surgery for Crohn's disease were excluded. Patient information, encompassing their medical history, clinical signs and symptoms related to Crohn's disease, laboratory diagnostic results, smoking status, prescribed treatments, and environmental conditions, was meticulously recorded.

### 2.1. Sample Collection

Stool samples were collected from the patients using sterile containers, with strict adherence to aseptic protocols. Subsequently, the levels of fecal Calprotectin were promptly measured using the CHORUS apparatus. Interpretation of the results was based on the following criteria: A result was considered POSITIVE when the level exceeded 60 mg/kg, and NEGATIVE when it fell below 40 mg/kg.

## 3. Results

### 3.1. Age Distribution of Study Patients

In the current study, a total of sixty Crohn's disease patients, comprising 30 smokers and 30 non-smokers, were included, as detailed in Table 1.

**Table 1** Age Groups Included in the Current Study.

Age group	Frequency of Smoking (n=30)	Percent	Frequency of Non-smoking (n=30)	Percent
10-29 Years	12	40%	4	13.4%
30-49 Years	16	54%	14	46.6%
50-60 Years	2	6.6%	10	33.4%
60 Years >	0	00%	2	6.6%
Total	30	100%	30	100%

### 3.2. The Mean±SD level of fecal calprotectin in cases of Crohn's disease

In this study, it was observed that the mean±SD concentration of fecal calprotectin levels in Crohn's disease patients was notably higher in the group of patients who smoked compared to those who did not, as illustrated in Table 2.

**Table 2** Mean±SD Concentration of stool calprotectin in case(smoking and nonsmoking) Crohn's disease.

Cases of Crohn's disease	NO	Mean±SD Level of Stool Calprotectin	P value
Smoking	30	125.21±23.63	0.00
Non-smoking	30	77.33±7.22	

### 3.3. Mean±SD concentration of fecal Calprotectin in different age groups

The mean±SD levels of fecal Calprotectin revealed variations across different age groups. Notably, the concentration of fecal Calprotectin was observed to be higher in the age group of smoking patients between 30 and 49 years. In contrast, lower levels were documented in the age group of non-smoking patients aged between 10 and 29 years.

**Table 3** Mean±SD concentration of Stool Calprotectin in different age groups.

Age group	Mean±SD Level of Stool Calprotectin	
	Smoking (n=30)	Non smoking (n=30)
10-29 Years	124.66±24.47	67.5±2.04
30-49 Years	125.62±22.97	77±5.95
50-60 Years	123±0	79.6±6.15
60 Years >	0	88±0

The analysis of stool Calprotectin concentration across various age groups yielded interesting findings. Among smoking patients, the age group of 30-49 years exhibited the highest concentration, with a mean±SD of 125.62±22.97. This was closely followed by the 10-29 years age group, which recorded a mean±SD of 124.66±24.47, while the age group of 50-60 years showed the lowest value, at 123±0.

Conversely, non-smoking patients displayed a different pattern. In this group, the age group of 50-60 years observed a slight increase in stool Calprotectin concentration, with a mean±SD of 79.6±6.15. This was preceded by the 30-49 years age group, with a mean±SD of 77±5.95, and the 10-29 years age group, with a mean±SD of 67.5±2.04.

#### 4. Discussion

Crohn's disease is characterized by an increased activation of immune cells, resulting in the release of pathogen-fighting proteins, such as calprotectin. The inflammatory process in the intestinal tract stimulates the proliferation and migration of neutrophil granulocytes into the intestinal lumen, leading to elevated levels of calprotectin in stool samples (26). The correlation between the presence of neutrophils in the intestinal lumen and increased stool calprotectin levels is particularly evident in inflammatory bowel diseases (IBD), including Crohn's disease (27-29). In our study, we observed significantly higher fecal calprotectin levels in smoking patients, while non-smoking patients showed only a slight elevation. This finding underscores the potential implication of smoking as a risk factor in the development of Crohn's disease and its impact on disease prognosis.

The concentration of stool calprotectin in Crohn's disease patients was notably elevated in tobacco smokers, with a mean±SD of 125.21±23.63, compared to non-smoking patients, who exhibited a mean±SD of 77.33±7.22. These results are in line with a previous study conducted by Costa, F., et al. in 2005 (30). More recent cohort studies have emphasized the significant role of environmental factors in the development of Crohn's disease (31-37). Furthermore, the mean±SD concentration of stool calprotectin revealed age-related variations, with the 30-49 years age group among smokers demonstrating the highest levels at 125.62±22.97, followed by the 10-29 years age group at 124.66±24.47, while the 50-60 years age group recorded the lowest value, at 123±0.

Interestingly, non-smoking patients in the 50-60 years age group exhibited a slight increase in stool calprotectin levels, with a mean±SD of 79.6±6.15. This was preceded by the 30-49 years age group, with a mean±SD of 77±5.95, and the 10-29 years age group, with a mean±SD of 67.5±2.04. These findings collectively suggest an age-dependent variation in stool calprotectin levels, with distinct patterns in smoking and non-smoking patients.

Numerous studies have implicated smoking as a significant risk factor in the development of Crohn's disease (38). The association between smoking and the prognosis of Crohn's disease has also been noted, with significantly higher risks of CD development in individuals who smoke (39). These findings underscore the complex interplay between environmental factors, smoking, age, and their influence on Crohn's disease, thereby contributing to a deeper understanding of the disease's pathogenesis and progression.

## 5. Conclusions

1. Elevated levels of stool calprotectin are significantly associated with smoking status, indicating a potential link between smoking and the pathophysiology of Crohn's disease.
2. Higher stool calprotectin levels in individuals who smoke may play a crucial role in predicting the prognosis of Crohn's disease, underscoring the relevance of calprotectin as a biomarker for disease management.
3. Smoking emerges as a direct and impactful environmental risk factor for bowel inflammation, highlighting the need to consider smoking cessation interventions in Crohn's disease management strategies.
4. To further elucidate the mechanisms underlying smoking-induced bowel inflammation, additional studies focusing on neutrophil proliferation within the intestinal lumen are recommended. This avenue of research may provide valuable insights into the interplay between smoking and Crohn's disease, facilitating more targeted therapeutic approaches and interventions.

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