



Neutrophil to Lymphocyte Ratio in Diagnosis of Inflammatory Bowel Disease and Correlation with Disease Severity Assessed by Ultrasound and Colonoscopy

Thesis

Submitted for partial fulfillment of Master Degree in
Internal Medicine

BY

Dina Salah khalil Mohamed

M.B.B.CH. – Ain Shams University

Under supervision of

Prof.Dr. Wesam Ahmed Ebrahim

Prof. of Internal Medicine and Gastroenterology
Faculty of Medicine, Ain Shams University

Prof.Dr. Shereen Abu Bakr Saleh

Assistant Prof. of Internal Medicine and Gastroenterology
Faculty of Medicine, Ain Shams University

Dr. Mohamed Osama Aly

Lecturer of Internal Medicine and Gastroenterology
Faculty of Medicine, Ain Shams University

**Faculty of Medicine
Ain Shams University
2019**

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

لَسْبَحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْعَظِيمُ

صدقة الله العظيم

سورة البقرة الآية: ٣٢



First and for most, thanks to Allah, whom I relate any success in achieving any work in my life.

I would like to extend my deep thanks and sincere appreciation to Prof. Dr. Wesam Ahmed Ibrahim Professor of Internal Medicine and Gastroenterology, Faculty of Medicine, Ain Shams University; for giving me this precious opportunity of working under her supervision; her vast knowledge and wide experience.

I would like to extend my deep thanks and sincere appreciation to Prof. Dr. Shereen Abu Bakr Saleh assistant professor of Internal Medicine and Gastroenterology, Faculty of Medicine, Ain Shams University; for her *great help, valuable advice and constant encouragement.*

I can't forget all many thanks to Dr. Mohamed Osama Aly Lecturer of Internal Medicine and Gastroenterology, Faculty of Medicine, Ain Shams University; for his kind support, meticulous supervisors, continuous encouragement and advising.

Dina Salah

LIST OF CONTENTS

Title	Page No.
LIST OF CONTENTS	I
LIST OF ABBREVIATIONS	II
LIST OF TABLES	IV
LIST OF FIGURES	VI
INTRODUCTION	1
AIM OF THE WORK	3
REVIEW OF LITERATURE	4
➤ Inflammatory Bowel Diseases	4
➤ Clinical presentation of IBD	9
➤ Clinical Assessment of IBD Activity	11
➤ Biomarkers to Assess IBD Activity	27
➤ Role of Neutrophils in IBD	31
➤ Effects of Targeting Neutrophil Chemotaxis and Extravasation	32
ENDOSCOPY IN IBD	36
MANAGEMENT OF IBD	41
PATIENTS AND METHODS	51
RESULTS	55
DISCUSSION	79
Conclusions and Recommendations	93
SUMMARY	94
REFERENCES	97
□ □ □ □ □ □ □ □	1

LIST OF ABBREVIATIONS

6-MP	6-mercaptopurine
ANCA	Anti-neutrophil cytoplasmic antibodies
ARDS	Acute respiratory distress syndrome
ASAs	Aminosalicylates
ASCA	Anti-Saccharomyces cerevisiae autoantibodies
AUC	Area under the curve
AZA	Azathioprine
cAMP	Cyclic adenosine monophosphate
CD	Crohn's disease
CDAI	Crohn's Disease Activity Index
CLE	Confocal laser endomicroscopy
CRP	C reactive protein
CT	Computed tomography
CTE	Computed tomography enterography
DBE	Double-balloon enteroscopy
ESR	Erythrocyte sedimentation rate
FC	Fecal calprotectin
FMT	Fecal microbiota transplantation
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HBD	Human defensin β
HLE	Human leucocytic elastase
IBD	Inflammatory bowel disease
IBDU	Inflammatory bowel disease unclassified
IL	Interleukin
IUS	Intestinal ultrasound
JAK	Janus kinase
MaRIA	Magnetic Resonance Index of Activity
MPO	Myeloperoxidase
MRE	Magnetic resonance enterography
MRI	Magnetic resonance imaging
MSCs	Mesenchymal stem cells
MTX	Methothrexate

LIST OF ABBREVIATIONS

NLR	Neutrophil-to-lymphocyte ratio
NPV	Negative predictive value
PDE	Phosphodiesterase
PPV	Positive predictive value
PSC	Primary sclerosing cholangitis
PUCAI	Pediatric Ulcerative Colitis Activity Index
ROC curve	Receiver operating characteristic curve
S1P	Sphingosine-1-phosphate
SBE	Single Balloon enteroscopy
SBFT	Small-bowel follow-through
SCCAI	Simple Colitis Clinical Activity index
SES-CD	Simple Endoscopic Score for Crohn's Disease
SMAD7	Mothers against decapentaplegic homolog 7
TNF	Tumor necrosis factor
TPMT	Thiopurine S-methyltransferase
UC	Ulcerative colitis
UCEIS	Ulcerative Colitis Endoscopic Index of Severity
VCE	Video capsule endoscopy
WBC	White blood cells

LIST OF TABLES

Table (1): Extraintestinal manifestations of inflammatory bowel disease.	10
Table (2): Truelove and Witts' Severity Index for use in Acute Severe Ulcerative Colitis	13
Table (3): Mayo Clinic Index: endoscopic subscore.....	15
Table (4): Bowel ultrasound severity score.....	18
Table (5): Crohn's Disease Activity Index (CDAI)	20
Table (6): Simple Endoscopic Score for Crohn's disease (SES-CD).....	22
Table (7): Gender differences in studied groups	55
Table (8): Age differences in studied groups	56
Table (9): NLR values in studied groups.....	57
Table (10): CRP values in studied groups.....	58
Table (11): ESR values in studied groups	59
Table (12): US trans-mural thickness in studied groups	60
Table (13): US trans-mural thickness in relation to activity degrees of IBD	61
Table (14): Relation of NLR to findings of ultrasound.....	62
Table (15): Gender differences in relation to activity degrees of IBD	63
Table (16): Age in relation to activity degrees of IBD.....	64
Table (17): NLR in relation to activity degrees of IBD.....	65
Table (18): showed that: There was significant correlation between CRP ($r=0.360$ $p=0.023$) and NLR but no significant correlation between NLR and ESR ($r=0.040$ $p=0.808$).	66
Table (19): ROC analysis of NLR in prediction of IBD activities.....	67

Table (20): US findings in different IBD activities.	68
Table (21): ROC curve of US findings in different IBD activities	69
Table (22): US coombs sign in different IBD activities.	70
Table (23): ROC curve of US coombs sign in detection of IBD activities.	71
Table (24): complications detected by US in different degrees of IBD activities.	72
Table (25): complications detected by us in different IBD activities.	73
Table (26):US lymph node enlargement in different IBD activities	74
Table (27): lymph node enlargement detected by US in different IBD activities.	75
Table (28): ROC analysis of NLR in patients with normal and those with abnormal US findings.	76
Table (29): ROC analysis of NLR in patients with normal and those with abnormal US findings.	77
Table (30): Relation between ultrasound findings and endoscopic scores of IBD activities.	78

LIST OF FIGURES

Fig (1): Domains of IBD disease activity assessment. Overlapping domains of disease activity assessment in IBD: inflammation, clinical symptoms, and quality of life (<i>Walsh et al., 2016</i>).....	11
Fig (2): Current and emerging therapies for inflammatory bowel disease (IBD).	42
Fig (3): Gender differences in studied groups.....	55
Fig (4): Age differences in studied groups.....	56
Fig (5): NLR values in studied groups	57
Fig (6): CRP values in studied groups	58
Fig (7): ESR values in studied groups.....	59
Fig (8): US trans-mural thickness in studied groups.....	60
Fig (9): US trans-mural thickness in relation to activity degrees of IBD.....	61
Fig (10): Relation of NLR to findings of ultrasound	62
Fig (11): Gender differences in relation to activity degrees of IBD	63
Fig (12): Age in relation to activity degrees of IBD	64
Fig (13): NLR in relation to activity degrees of IBD	65
Fig (14): ROC analysis of NLR in prediction of IBD activities.	67
Fig (15):US findings in different IBD activities.....	68
Fig (16): ROC curve of US findings in detection of IBD activities.....	69
Fig (17):US coombs sign in different IBD activities.....	70
Fig (18):ROC curve of US combs sign in detection of IBD activities.	71
Fig (19):complications detected by us in different IBD activities.....	72
Fig (20): ROC curve of complications detected by US in different IBD activities...	73
Fig (21):US lymph node enlargement in different IBD activities.	74
Fig (22): ROC curve of LN enlargement detected by US in different IBD activities.	75
Fig (23): ROC analysis of NLR in patients and control groups.	76
Fig (24): ROC analysis of NLR in patients with normal and those with abnormal US findings.....	77
Fig (25):Ccorrelation between ultrasound findings and endoscopic scores of IBD activities	78

Abstract

Background: Inflammatory bowel disease (IBD) is a chronic idiopathic disease affecting the gastrointestinal tract that is comprised of two separate, but related intestinal disorders; Crohn's disease (CD) and ulcerative colitis (UC). The diagnosis of IBD is most commonly made on the basis of clinical features in combination with findings on ultrasonography, endoscopy and histopathology. **Patients and Methods:** This is a cross-sectional prospective observational study. This study included 40 IBD patients (19 females and 21 males), presented to Internal Medicine department, El-Demerdash Hospital, Ain Shams University from June 2018 to June 2019. Patients were matched with 10 healthy subjects (7 females and 3 males) as a control group. All patients subjected to the following: Thorough medical history, clinical examination (general and local) and laboratory investigations including complete blood count with calculation of neutrophils to lymphocyte ratio, ESR and CRP on the day of the colonoscopic procedure. US examination and endoscopic examination were done for every patient. **Results:** Mean value of NLR in IBD patients was significantly higher than those in control group representing 3.12 ± 1.87 and 1.88 ± 0.42 respectively but it was statistically insignificant between active and inactive IBD patients with best cut off >2.01 to differentiate active from inactive patient. THE NLR value was correlating with CRP only with no correlation with ESR, NLR value also has association with US findings that suggesting activity with best cut off >2.5 to detect these findings by US in active patients. US has high sensitivity and specificity in discriminating patients with moderate and severe activity from those with mild activity or those in remission but it was unable to discriminate patient of moderate activity from patients with severe activity with strong association with endoscopic scores of activity. **Conclusion:** NLR values had higher sensitivity and specificity than CRP and ESR with the benefit of being readily available with an affordable price. They are more useful when utilized together with serum laboratory inflammatory indices (CRP and ESR).

Key words: neutrophil, lymphocyte, inflammatory bowel disease, ultrasound, colonoscopy

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammatory disorder of the alimentary tract that mainly comprised of ulcerative colitis (UC), Crohn's disease (CD), and inflammatory bowel disease unclassified (IBDU). It is chronic and incurable, and presents with periods of relapse and remission. IBD etiology is unknown, but it has been postulated to be a multifactorial disease due to the genetic, immunologic, and environmental factors involved in its development (**Yamamoto-Furusho et al., 2017**).

Ulcerative colitis (UC) is a chronic subtype that diffusely involves various parts of the colon. The disease may be limited to the rectum, or it may involve the entire colon. The main complaints of the patients are bloody diarrhea and abdominal pain (**Singh et al., 2016**). Mucosal examination via colonoscopy is the basic method in diagnosis; it monitors the activity of the disease and is used in the follow-up of patients (**Akpinar et al., 2018**).

Crohn's disease (CD) is chronic relapsing and remitting another subtype of IBD, with an unknown etiology and appears to involve interaction between genetic susceptibility, environmental factors and the immune system. Previous studies suggested that early detection of disease activity could significantly reduce the mortality of CD (**Gao et al., 2015**).

Colonoscopy is the basic diagnostic and therapeutic modality in ulcerative colitis. However, colonoscopy may not be always feasible in these patients. Oversensitivity to air insufflations in patients with active ulcerative colitis during the procedure, or unavailability of colonoscopy necessitates the use of different parameters to evaluate the patients **(Makkar, 2013)**.

Non-invasive tests, such as C reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cells (WBC), acid glycoprotein, platelet count and albumin are therefore being increasingly recognized as important markers for initial diagnosis and disease activity detection **(Mack et al., 2007)**.

In recent years, the neutrophil-to-lymphocyte ratio (NLR), calculated as total neutrophil count divided by total lymphocyte count, has gained increased attention for assessing the grade of inflammation. The NLR was initially described as a general immunological response to different stress stimuli, which correlated with outcomes and organic dysfunction scores of critically ill intensive care unit patients **(Argeny et al., 2018)**.

Recent reports revealed the predictive value of NLR in discriminating active from inactive UC, compared to the mixed results regarding the diagnostic value of CRP alone **(Celikbilek et al., 2013; Posul et al., 2015)**

AIM OF THE WORK

The aim of the present study was to evaluate the neutrophil to lymphocyte ratio as simple rapid and cheap test readily available for assessment of severity of IBD findings of ultrasonography and colonoscopy.

INFLAMMATORY BOWEL DISEASES

Inflammatory bowel disease (IBD) comprises two chronic intestinal disorders: ulcerative colitis (UC) and Crohn's disease (CD). These disorders were first described by Sir Samuel Wilks in 1859 and Doctor Crohn in 1932, respectively. CD and UC are characterized by a course of remission and relapse with complex interactions among genes, the environment, and immunity (**Ananthakrishnan, 2015**).

Incidence and prevalence of IBD

It is estimated that 1.5 million in North America and 2.5 million persons in Europe have IBD. The peak incidence of CD and UC is between 20–30 years and 30–40 years of age, respectively (**Arora and Malik, 2016**).

The incidence and prevalence of CD and UC have stabilized in the aforementioned regions; however, it is still higher than in the rest of the world (**Ananthakrishnan, 2015**).

IBD have always seemed to be rare in the Middle East and Northern Africa. No accurate registry or cohort of patients had ever studied the exact prevalence of CD and UC in these populations. In Mediterranean countries, the prevalence of UC was estimated at 5/100000 in urban areas. The incidence of IBD

seems to be rising in Egypt with UC to CD ratio of 6:1 (**Esmat et al., 2014**).

Age and gender disparity

Although IBD can occur at any age, the peak age of onset for CD and UC is generally between 20–30 years and 30–40 years of age, respectively. However, some European cohorts have suggested a second peak between 60–70 years of age, especially for UC. The most plausible explanation for this additional peak is ascertainment bias due to increased health care access and more frequent evaluation of older patients. Majority of North American population-based study has shown that the median and mean age of diagnosis of CD and UC range between 30–45 years and 40–45 years, respectively. Additionally, these studies especially in adults have suggested a female predominance in CD and male predominance in UC (**Ananthakrishnan, 2015**).

Racial and ethnic disparity

There appears to be a marked ethnic and racial variation in the incidence of IBD. Early studies from the 1960s reported a lower incidence of IBD, specifically UC among African-Americans. However, these studies were conducted in regions with predominant white populations, and more recent studies from 1990s have challenged these findings with comparable incidence rates among Whites and non-Whites. Further, CD was proposed to