Zagazig University Medical Journal
www.zumj.journals.ekb.eg

Volume 28, Issue 4, July 2022(748-761)

Manuscript ID ZUMJ-2010-1983 (R3)
DOI **10.21608/zumj.2020.47756.1983**

ORIGINAL ARTICLE

P53&AMCR Over-expression Accelerate Dysplastic Progression in CMV Ulcerative Colitis Patients (A Comparative Study of CMV and Non-CMV Ulcerative Colitis)

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Submit Date 2020-10-27
Revise Date 2020-11-26
Accept Date 2020-12-07

ABSTRACT

Background: CMV Virus is one of the most common opportunistic infections in ulcerative colitis patients that leads to more immunological and inflammatory irritation and thus leads to treatment resistance and dysplastic progression. This work aims to evaluate the role of P53 & AMACR as early predictor markers of dysplastic transformation and clinical deterioration in CMV-infected ulcerative colitis patients.

Method: Forty CMV-ulcerative colitis and twenty Non-CMV-ulcerative colitis patients with active colitis underwent baseline assessment for clinical endoscopic evaluation, histological evaluation of the degree of inflammation, and dysplasia, and P53/AMACR expression detection. Cases were then classified into four groups, namely, CMV-UC with P53/AMACR, CMV-UC without P53/AMACR, Non-CMV-UC with P53/AMACR, and Non-CMV-UC without P53/AMACR. After 36.16±3.78 months of follow-up, the same assessment was carried out to record the progression parameters of all groups.

Results: CMV-UC with P53&AMACR group showed a significant association with clinical, and histological progression 8/22 (36.4%) in compared with CMV-UC without P53/AMACR and the clinical and histological progression was 1/18 (5.6%) and 2/18 (11.1%) respectively and in Non-CMV-UC with P53/AMACR group were 1/9 (11.1%) and 2/9 (22.2%) respectively with (P-value<0.001**)

Conclusions: P53/AMACR co-expression is an early indicator of dysplastic progression, treatment resistance, and clinical deterioration. Patients with UC should have a regular examination for CMV infection and early CMV treatment before mutations of p53 and AMACR are overexpressed, as their presence reduces the chances of recovery and accelerates dysplastic progression.

Keywords: P53; AMACR; CMV; dysplasia; ulcerative colitis

INTRODUCTION

Ulcerative colitis (UC) is a type of chronic inflammatory bowel disease (IBD) that involves the rectum and colon characterized by alternating periods of exacerbation and remission. The treatment of UC differs according to its activity and extent [1]. The risk of colorectal cancer (CRC) is 2-5 times higher in patients with ulcerative colitis (UC) than in the general population, and it is a major cause of morbidity and mortality among these individuals.[2]

Acute severe ulcerative colitis affects up to 25% of patients, either on the first presentation or later, and requires hospital admission for treatment with intravenous steroids. About 30% of these patients are resistant to steroid therapy and colectomy is the usual option [3],

Conventional therapies are in many instances ineffective or cannot be tolerated by the patients. This resistance to treat UC patients is apparent in the frequency of colectomies performed; the cumulative probability of colectomy from the time of diagnosis

is 13.1% at 5 years, 18.9% at 10 years, and 25.4% at 20 years [4].

Cytomegalovirus (CMV), a member of the Herpes-viridae family, is responsible for a common viral infection in humans, with 40 – 100 % of adults exhibiting stigmata of past infection. CMV persists in a latent form throughout the lifetime of the infected subject and viral replication can be reactivated, especially in situations of immunosuppression such as organ transplantation and immunosuppressive treatment.[5]

The link between cytomegalovirus (CMV) infection and inflammatory bowel diseases remains an important subject of debate. CMV infection is frequent in ulcerative colitis (UC) whereas the prevalence of CMV colitis in resected IBD specimens ranges from 17.9% to 22% and 27% in steroid refractory UC patients and is potentially harmful [6]. However, CMV infection in UC may appear de novo or as an opportunistic infection on top of steroids and immunosuppressive drugs, some treatments, notably steroids and cyclosporine A, have been shown to favor reactivation of a latent CMV, which can be considered as a cause of active flare-ups of refractory UC, [7]

Infection of host cells with human cytomegalovirus (HCMV) induces cell cycle dysregulation through the cellular accumulation of high levels of p53 protein in HCMV-infected cells. HCMV (IE) proteins in HCMV-infected cells prevent p53 binding to p53-specific DNA sequences, resulting in multiple outcomes, such as stimulation of cellular DNA synthesis, cell cycle progression , cell cycle arrest, and prevention of programmed cell death. [8]

In UC associated cancer and dysplasia, the prevalence of p53 protein overexpression has been reported to be 71 to 100% and 30 to 80%, respectively. Thus, the immunohistochemical detection of p53 protein overexpression can be utilized as a signal of neoplastic change or for a more objective differential diagnosis of neoplastic progression and/or dysplasia from non-neoplastic conditions.[9]

This work aims to evaluate the combined role of P53 & AMACR expression as early predictor markers of dysplastic transformation, treatment resistance, and clinical progression in CMV infected ulcerative colitis patients

METHODS

60 patients with UC are the subject of this study. These patients are treated and annually followed up at the Departments of Tropical Medicine and Internal Medicine with associated Gastroenterology, Hepatology, and endoscopy unit of the faculty of medicine, Zagazig University, Egypt, between January 2012 and March 2016. The diagnosis of UC was made by previously established international criteria based on clinical, endoscopic, histopathological, and radiological findings.[11] Written informed consent was obtained from all participants, the study was approved by the research ethical committee of the Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans

Patients' classification

Forty CMV-ulcerative colitis and twenty Non CMV-ulcerative colitis patients with active colitis grade I According to Riddell's criteria [15] underwent baseline assessment for clinical endoscopic evaluation, histological evaluation of the degree of inflammation and dysplasia, and P53/AMACR expression detection. Cases were then classified into four groups according to the presence and absence of P53/AMACR, namely, CMV-UC with P53/AMACR, CMV-UC without P53/AMACR, Non CMV-UC with P53/AMACR, and Non CMV-UC without P53/AMACR

After 36.16±3.78 months of follow-up the same assessment was carried out to record the progression parameters of all groups

CMV infection was detected in colonic tissue by RT-qPCR at baseline, assessment, and follow-up assessment

Baseline assessment:

Clinical evaluation:

All patients are evaluated for (clinical stage) through Endoscopic Score evaluation using Hanauer's Sigmoidoscopic Index [11]

Histological evaluation:

Evaluation of inflammation using the modified Riley Score [14]

Evaluation of dysplasia using Riddell's criteria [15]

Immunohistochemically and Tissue RT-qPCR for P53 expression,AMACR expression

Patient follow-up:

All patients are under medical treatment and supervision of staff members of the tropical and internal medicine departments. CMV-UC patients started administration of intravenous ganciclovir at 5 mg/kg/12 h for 3 weeks.[12] as a treatment of CMV infection after detection during the first assessment.

The mean duration of follow-up was 36.16±3.78 months from the first assessment. During the study period, colonoscopies were done once every 12 months as a regular clinical follow-up at the gastroenterology, hepatology, and endoscopy unit.

Follow-up assessment:

After a mean of 36.16±3.78 months, follow-up assessment was done by applying the same protocol of the baseline assessment,

After the follow-up evaluation parameters of progression was carried out by comparing the results of the first assessment with the second assessment

Parameters of outcome (progression index):

Clinical outcome: any case showing an increase of Hanauer's Sigmoidoscopic Index is considered clinical progression (deterioration), while no change is considered stationary and a decrease in Hanauer's Sigmoidoscopic Index would be considered regression (improvement)

Histological outcome: cases showing an increase of both modified Riley's score (inflammatory score) and Riddell's score (dysplastic score) are considered histological progression (deterioration) while a decrease in both indices denotes regression (improvement)

Marker outcome: cases showing an increase of both P53 score and AMACR score by both IHC or RT-qPCR are considered marker progression, while a decrease in both markers represents regression.

CMV reactivation: this is assessed by the detection of CMV in tissue by RT-qPCR after follow-up assessment

Clinical study (Endoscopic Evaluation):

Records of enrolled patients include sex, age at diagnosis of UC, and duration of UC. All patients received the same bowel preparation (magnesium citrate). During index colonoscopy, the colon was divided into 8 segments (cecum, ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon, sigmoid colon, and rectum). Biopsies from each segment were taken using the protocol of the recommended 4- quadrant biopsies every 10 cm of the colon and 5 cm of the rectum.[13] biopsies from each location were preserved in 10% formalin for further histological and immunohistochemical study and, Aliquots of each tissue specimen were preserved in RNA*later* solution and stored at −80° C for further molecular work

We detect the clinical-stage Using Hanauer's Sigmoidoscopic Index: it is composed of five variables (Figure 1)

Erythema, (0, normal; 1, mild; 2, moderate; 3, severe), Friability, (0, normal; 1, mild; 2, moderate; 3, severe), Mucopus, (0, normal; 1, mild; 2, moderate; 3, severe), Granularity/ulceration, (0, normal; 1, mild; 2, moderate; 3, severe), Disappearance of the mucosal vascular pattern (0, normal; 1, mild; 2, moderate; 3, severe).

Each variable was assigned a value from 0 to 3 (0, normal; 1, mild; 2, moderate; 3, severe). Total scores for the Sigmoidoscopic Index ranged from 0 to 15 points. [1] All five endoscopic scores were measured for each patient and staged as follows: Score 1-5, stage 1, Score 6-10 stage 2, Score 11-15, stage 3

A subset of 10 patients had their endoscopy images rescored by a second blinded endoscopist to determine the interobserver agreement for the endoscopic score. A substantial agreement between endoscopists was reached.

Histological Study:

The biopsies were fixed in 10% formalin and embedded in paraffin, all paraffin blocks were cut into three serial 3-µmthick slices for hematoxylin and eosin (HE) staining. All slides are derived from the pathology department, Zagazig University, where it is evaluated and scored by a panel of three different gastrointestinal pathologists blinded to the patient's disease status and endoscopic scores.

Two types of histological evaluation were done

-Evaluation of inflammation according to the Modified Riley Score [14]

-Evaluation of dysplasia according to Riddell's criteria [15]

Evaluation of inflammation:

According to The Acute Inflammation Subscale of the modified Riley Score with Conversion to 4-tiered Grading System [14].

No activity (no extravascular neutrophils) Grade 0; Normal biopsy or inactive colitis, Mild activity (lamina propria neutrophils only) Grade I include: Scattered individual neutrophils score 1, Patchy collections of neutrophils (score 2), and diffuse neutrophilic infiltrate (score 3). Moderate activities (cryptitis/crypt abscesses) Grade 2 include: <25% of crypts involved (score 4), 25%–74% of crypts involved (score 5), >75% of crypts involved (score 6). Severe activity Grade 3 include: Erosions and ulcers (score 7).

Evaluation of dysplasia:

According to Riddell's criteria for Classification of dysplasia of IBD.[15] (figure 3)

Negative with score 0; Normal mucosa, Inactive colitis (quiescent colitis), and active colitis,

Indefinite dysplasia with score 1: Probably negative (probably inflammatory), unknown, and Probably positive (probably dysplastic). Positive with score 2: Low-grade dysplasia. Positive with score 3: High-grade dysplasia

Immunohistochemical Study :

Immunohistochemistry was performed on tissue fixed with 10% neutral buffered formalin, then embedded in paraffin and sectioned into 4-µm slices. Immunostaining was carried out using a Leica BOND-MAX™ autostainer (Leica GmbH, Nussloch, Germany), according to the manufacturer's protocol. Slides were dewaxed in Bond™ Dewax Solution (Leica Microsystems) and rehydrated in Bond Wash Solution (Leica Microsystems). Antigen retrieval was performed at pH 6 using Bond Epitope Retrieval 1 Solution (Leica Microsystems) for 30 min at 100 °C. Slides were incubated for 20 min at room temperature with monoclonal primary antibodies against (CMV, P53, and AMACR)

P53: (Thermo SCIENTIFIC: p53 (SP5) Rabbit Monoclonal Antibody, Catalog # RM-9105-S0, -S1, or –S, at 1:100 dilution)

AMACR: (Thermo SCIENTIFIC: p504S/ AMACR (Clone 13H4) Rabbit Monoclonal Antibody Cat. #RM-9130-A0, -A1, or -A Purified with BSA and Azide, at 1:100).

Primary antibody binding to tissue sections was visualized using biotin-free Bond Polymer Refine Detection (Leica Microsystems). After postprimary amplification (8 min; Leica Microsystems) and detection with the Novolink Polymer Detection System (15 min; Leica Microsystems) using 3,3′-diaminobenzidine (Novocastra Laboratories; 1:50), slides were counterstained with hematoxylin (Leica Microsystems).

Positive IHC reactions were defined as brown granular cytoplasmic pattern staining for AMACAR and a positive nuclear brown reaction for P53

Immunohistochemical Scoring

Scoring of P53

P53 protein overexpression was classified as: [9] (−), negative < 5%, (+), a few scattered positive cells 5%to14%,(++), localized aggregation of positive cells 15% to 50%, (+++), diffusely positive cells > 50%

Scoring of AMACR

All immunohistochemical staining results were evaluated semiquantitatively as follows [18]

AMACR expression intensity was scored: Negative, 0 (No cytoplasmic or granular staining), Weak 1 (faint diffuse cytoplasmic or granular apical staining), Moderate 2 (mild granular cytoplasmic staining),

Strong 3 (intense granular cytoplasmic stain). Staining extent was rated according to the percentage of positive cells: cases with

< 5% of lesional cells staining positive, were scored as (0)

5%to14% of lesional cells staining positive were scored as (1+)

15% to 50% of lesional cells staining positive, were scored as (2+)

> 50% of lesional cells staining positive, were scored as (3+).

The score of staining intensity multiplied by the score of extent equals an overall staining score. An overall staining score of

0, negative

1-3 weak

4-6 moderate

7-9 strong

Molecular Study:

CMV, P53, and AMACR evaluation in tissue using real-time PCR (Figure 2)

CMV detection in tissue using real-time qPCR:

For detection of CMV DNA in patient samples, DNA was extracted from the frozen biopsy sample fragments using Qiamp DNA mini kit following the manufacturers' instructions. The starting material was 5-25 mg from each sample. A carrier RNA was added to each sample in the lysis step to ensure optimal binding conditions for samples with low viral DNA concentration. CMV DNA was qualitatively identified in the extracted DNA of each sample using gene proof Cytomegalovirus (CMV) PCR Kit. A provided internal standard control was added to each sample during the lysis step for validation of extraction and amplification procedures. The reaction mix and thermal profile were performed as per the manufacturer's instructions. The target DNA was detected in the FAM channel while the internal standard was detected using the HEX channel. The reaction was performed using Step one applied biosystem QPCR System, Agilent technologies. A positive standard control (provided with the kit) and Negative (No template) control samples were included in each run.

Quantitative determination of P53 and AMACR genes' expression.

Total RNA from the samples was obtained using the RNeasy Mini Kit (Qiagen, Inc., Germany). Approximately 20 mg from each frozen sample was

used. The isolated total RNA concentration was measured using (Quantus™ Fluorometer, Promega, USA) and 0.5 ug from each sample was directly reverse transcribed using High-Capacity cDNA Reverse Transcription Ki (Applied biosystem). Reverse transcription was performed at 25ºC for 10 min and then at 37ºC for 120 min. mRNA copy numbers of p53, Alpha-methyl acyl CoA racemase AMACR, and GADPH as a housekeeping gene were determined in each sample by real-time quantitative RT-qPCR using Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific) on Step one applied biosystem QPCR System, Agilent technologies. The amplification mix with a total volume of 25 ul included 12.5 ul from the SYBR Green master mix, 10 nM from rox solution, 0.5 uM from each primer, 5 ul from cDNA, and the rest of the volume water. Amplification started with a 10 min. Denaturation step at 95ºC followed by 45 cycles of denaturation at 95ºC for 15 s, annealing at 60ºC for 30 s, and extension at 72ºC for 40 s. Data acquisition was performed during the extension step. A dissociation curve was done after the end of cycles to check for reaction specificity, in which a single melting point peak was ensured for each gene amplification product. The relative expression of each gene was determined using the ΔΔ Ct method with the endogenous GADPH as a control to normalize and quantitatively compare samples. The primer sequences used were as following:

p53: Forward primer, 5'-GAGCTGAATGAGGCCTTGGA-3′

Reverse primer, 5' -CTGAGTCAGGCC CTTCTGTCT T-3′

GAPDH: Forward primer, 5' -TGATGACATCAAGAAGGTGGTGAA-3′

Reverse primer, 5'-TCCTTGGAGGCCATGTGGGCCAT-3′

AMACR: Forward primer, 5′-GGGTCAGGTCATTGATGCAAA-3′

Reverse primer, 5′-TTCCCACAGACTCAATTTCTGAGTT-3′

Negative control was included in each run to access specificity of primers and possible contamination.

Quantification of target gene expression

The relative quantification of *p53 and AMACR* genes was calculated using the following formula:

Ratio = (a/b) / (c/d) where

a: measured expression of (*p53or AMACR)* gene in the tumor sample

b: measured expression of the housekeeping gene, *GAPDH* in the tumor sample

c: measured expression of (*p53 or AMACR)* gene in the normal sample

d: measured expression of the housekeeping gene, *GAPDH* in the normal sample

The PCR efficiencies for the amplification of the specified gene and *GAPDH* genes were calculated using the following formula: PCR efficiency = $10^{-1/slope}$

Statistical analysis

Statistical presentation and analysis of the present study were conducted, using the mean, Standard Deviation, and chi-square tests by (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

The hypothesis is that the row and column variables are independent, without indicating the strength or direction of the relationship. Pearson chi-square and likelihood ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables.

>0.05 Non significant <0.05* significant <0.001** High significant

RESULTS

Basic characteristics

T1he study included 60 patients with ulcerative colitis. 40 patients with CMV ulcerative colitis and 20 patients with Non-CMV ulcerative colitis. Males constituted 55% while females constituted 45% of the studied patients. The mean age at diagnosis of UC was 29.8±5.8 years and ranged from 20 to 42 years. (Table 1).

Comparison between baseline assessment and follow-up assessment of patients with CMV-ulcerative colitis and Non-CMV-ulcerative colitis according to the presence of P53&AMACR expression (Table 2).

Modified Riley score (inflammatory index)

In CMV-UC without P53&AMACR group :

At baseline assessment, grade1, grade2, and grade3 constituted (11, 7, 0) of the studied patients, while after follow-up constituted (14, 13, 1) of the studied patients respectively. This means that in this group we had 3/18 patients with regressive course from grade2 to grade1 and only 1/18 case with a progressive course from grade2 to grade3 and 14/18 patients with a stationary course

In CMV-UC with P53&AMACR group

At baseline assessment, grade1, grade2, grade3 constituted (15, 6, 1) of the studied patients, while after follow-up constituted (9, 12, 1) of the studied patients respectively. This means that in this group we had 6/22 cases with a progressive course, 6 cases

from grade1 into grade2, and 16/22 patients with a stationary course

In Non CMV-UC without P53&AMACR group

At baseline assessment, grade1, grade2, and grade3 constituted (6, 5, 0) of the studied patients, while after follow-up constituted (9, 2, 0) of the studied patients respectively. This means that in this group we had 0/11 patients with a progressive course, and 3/11 patients with regressive course from grade2 to grade1, and 6/11 stationary case.

In Non-CMV-UC with P53&AMACR group

At baseline assessment, grade1, grade2, grade3 constituted (4, 5, 0) of the studied patients, while after follow-up constituted (5, 3, 1) of the studied patients respectively. This means that in this group we had 1/9 patients with regressive course from grade2 to grade1 and 1/9 cases with progressive course from grade2 into grade3 and 7/9 patients with a stationary course

Riddell's score (dysplastic index)

among 60 cases of active ulcerative colitis, after follow-up, there were two cases turned into colorectal carcinoma that was among CMV-UC with P53&AMACR group, as well as there was one case turned to high-grade dysplasia were within the same group. As for the cases turned from colitis into low-grade dysplasia, there were four cases, two of them were in the same group (CMV-UC with P53&AMACR) and one was in the CMV-UC without P53&AMACR group and the other followed the Non CMV-UC with P53&AMACR group, As for the cases that turned from colitis into indefinite dysplasia, there were 6 cases, 3 of them were also within CMV-UC with P53&AMACR and one in each of the other 3 groups,

Hanauer's Sigmoidoscopic index (clinical index)

In CMV-UC without P53&AMACR group :

At baseline assessment, grade1, grade2, grade3 constituted (11, 7, 0) of the studied patients, while after follow-up constituted (15,2, 1) of the studied patients respectively. This means that in this group we had 4/18 patients with a regressive course from grade2 to grade1 and 1/18 cases with a progressive course from grade 2 to grade 1 and 13/18 case with stationary course

In CMV-UC with P53&AMACR group

At baseline assessment, grade1, grade2, grade3 constituted (12, 9, 1) of the studied patients, while after follow-up constituted (8, 9, 5) of the studied patients respectively. This means that in this group we had 8/22 cases with progressive course 4cases from grade1 to grade2 and 4 cases from grade2 to grade 3 and 14/22 patients with a stationary course

In Non CMV-UC without P53&AMACR group

At baseline assessment, grade1, grade2, grade3 constituted (7,4, 0) of the studied patients, while after follow-up constituted (9, 2, 0) of the studied patients respectively. This means that in this group we had 2/11 patients with a regressive course from grade2 to grade1 and 9/11 patients with a stationary course

In Non CMV-UC with P53&AMACR group

At baseline assessment, grade1, grade2, grade3 constituted (3, 6, 0) of the studied patients, while after follow-up constituted (4, 4, 1) of the studied patients respectively. This means that in this group that we had 1/9 patients with progression from grade2 to grade3, 1/9 patient with regression course, from grade2 to grade1, and 7/9 cases with stationary course

Comparison between CMV-ulcerative colitis with P53&AMACR group and CMV-ulcerative colitis without P53&AMACR group (Table 3)

Clinical outcome

In CMV-UC with P53&AMACR group, There was a significant association with clinical progression where 8/22 (36.4%) patients showed clinical progression after follow-up assessment compared with 1/18 (5.6%) of CMV-UC with P53&AMACR with (P-value<0.001**)

Histological outcome

In CMV-UC with P53&AMACR group, There was a significant association with histological progression where 8/22 (36.4%) patients showed histological progression after follow-up assessment compared with 2/18 (11.1%) of CMV-UC with P53&AMACR with (P-value<0.001**)

Markers outcome

In CMV-UC with P53&AMACR group, There was a significant association with marker progression where 10/22 (45.5%) patients showed marker progression after follow-up assessment compared with 1/18 (5.6%) of CMV-UC with P53&AMACR with (P-value<0.001**)

CMV outcome

In CMV-UC with P53&AMACR group, There was a significant association with CMV reactivation where 9/22 (40.9%) patients showed CMV reactivation after follow-up assessment compared with 1/18 (5.6%) of CMV-UC with P53&AMACR with (P-value<0.001**)

https://dx.doi.org/10.21608/zumj.2020.47756.1983
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Table (1): Basic characteristics of 60 patients with ulcerative colitis

		All patients (n=60)
Sex		
Male		33(55%)
Female		27(45%)
Age at diagnosis of UC (years)		
Range		20-42
Mean±SD		29.8±5.8
Duration of UC (years)		
	Range	Mean±SD
CMV-UC without P53&AMACR (n=18)	16-24	20±2.8
CMV-UC with P53&AMACR (n=22)	17-26	21±3.9
Non CMV-UC without P53&AMACR (n=11)	17-22	19±1.4
Non CMV-UC with P53&AMACR (n=9)	17-24	20±2.6

Table (2): Comparison between baseline assessment and follow-up assessment of patients with CMV-ulcerative colitis and Non-CMV-ulcerative colitis according to the presence of **P53&AMACR** expression

	CMV-UC (n=40)				**Non CMV-UC (n=20)**				
	Without P53&AMACR (n=18)		**With P53&AMACR (n=22)**		**Without P53&AMACR (n=11)**		**With P53&AMACR (n=9)**		
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	
Modified Riley score									
Grade 1	11	14	15	9	6	9	4	5	
Grade 2	7	3	6	12	5	2	5	3	
Grade 3	0	1	1	1	0	0	0	1	
Riddells score									
Active colitis	18	16	22	14	11	10	9	7	
Indefinite dysplasia	0	1	0	3	0	1	0	1	6
Low grad dysplasia	0	1	0	2	0	0	0	1	4
High grade dysplasia	0	0	0	1	0	0	0	0	1
Colorectal carcinoma	0	0	0	2	0	0	0	0	2
Hanauer's Sigmoidoscopic index									
Stage 1	11	15	12	8	7	9	3	4	
Stage 2	7	2	9	9	4	2	6	4	
Stage 3	0	1	1	5	0	0	0	1	

Table 3: Comparison between CMV-ulcerative colitis with the P53&AMACR group and CMV-ulcerative colitis without the P53&AMACR group

	CMV-UC without P53&AMACR (n=18)		CMV-UC with P53&AMACR (n=22)		Chi-square
	N	%	N	%	P-value
Clinical outcome					
Regression	17	94.4	14	63.6	<0.001**
Progression	1	5.6	8	36.4	
Histological outcome					
Regression	16	88.9	14	63.6	<0.001**
Progression	2	11.1	8	36.4	
Markers outcome					
Regression	17	94.4	12	54.5	<0.001**
Progression	1	5.6	10	45.5	
CMV outcome					
Remission	17	94.4	13	59.1	<0.001**
Reactivation	1	5.6	9	40.9	

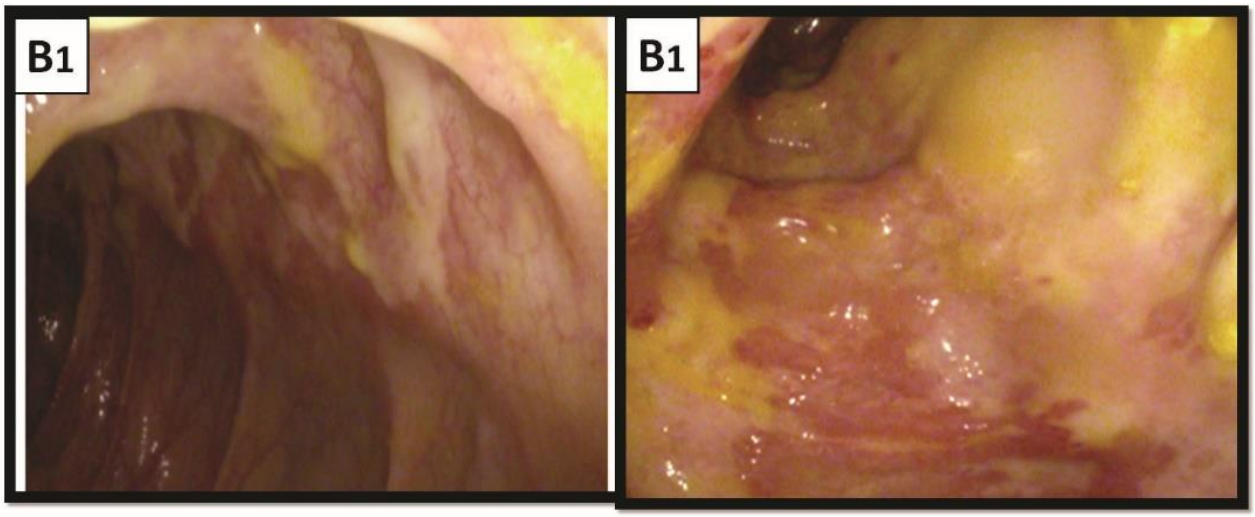

Figure 1.A1: Colonoscopy of descending colon showing superficial ulceration , patchy obliteration of vascular pattern, no evidence of bleeding indicating mild to moderate ulcerative colitis. Hanauer's Index score 4 (baseline assessment). A2: Colonoscopy of rectum showing erosions & superficial large ulcerations, with loss of vascular pattern and hemorrhagic spots indicating ulcerative colitis of severe activity. Hanauer's Index score 11 (follow-up assessment).

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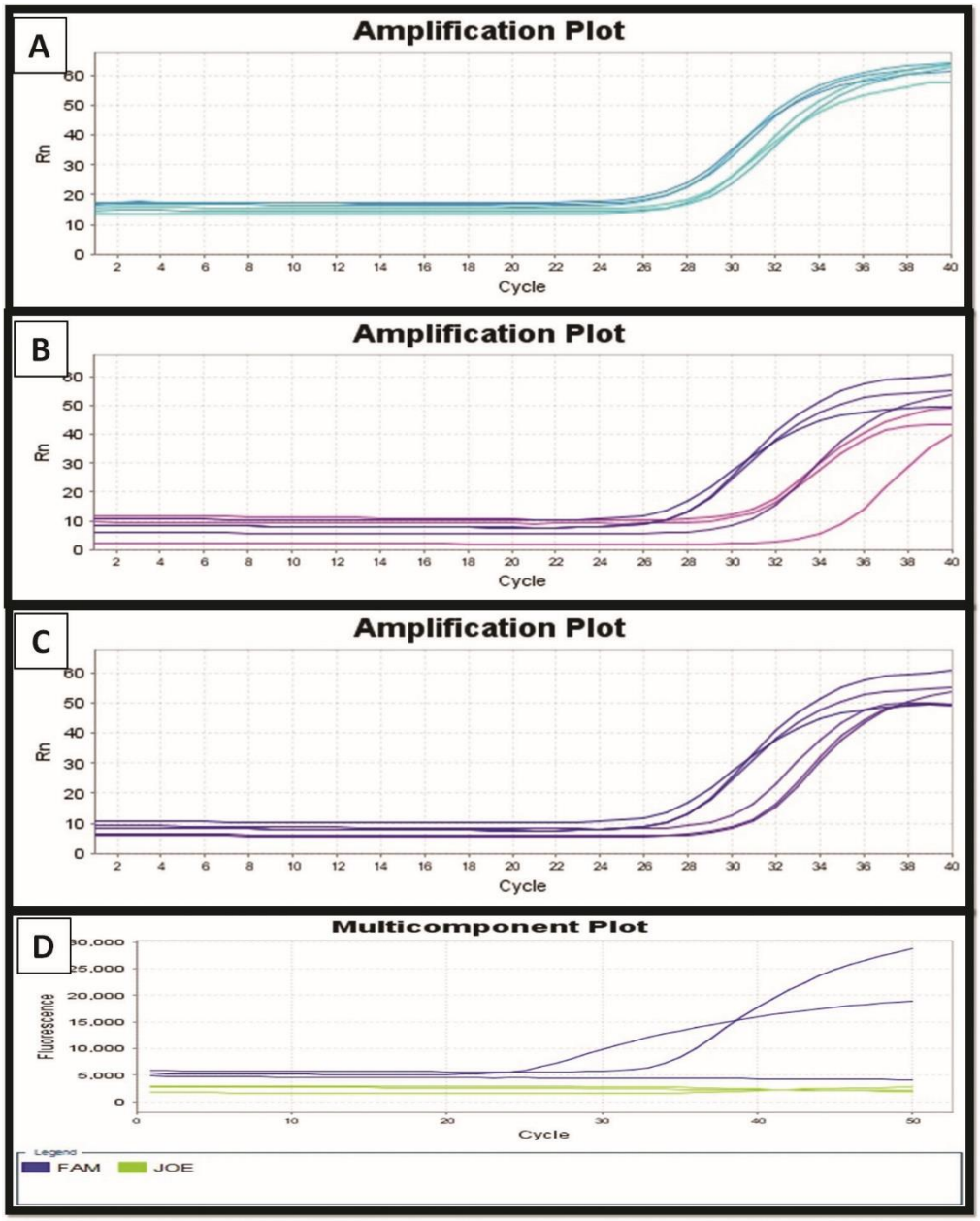

Figure 2. Images (A, B, C, D) illustrating Amplification plots for (GADPH, P53, AMACR, CMV). A: illustrating Amplification plots for GAPDH, by real-time PCR using sybrgreen mastermix.B: Amplification plots for p53 by real-time PCR using sybrgreen mastermix. C: Amplification plots for AMACR by real-time PCR using sybrgreen mastermix. D: Multicomponent Plot for CMV (FAM) in blue and Internal control (Joe) in green by real-time PCR using TaqMan fluorogenic system (geneproof kit)

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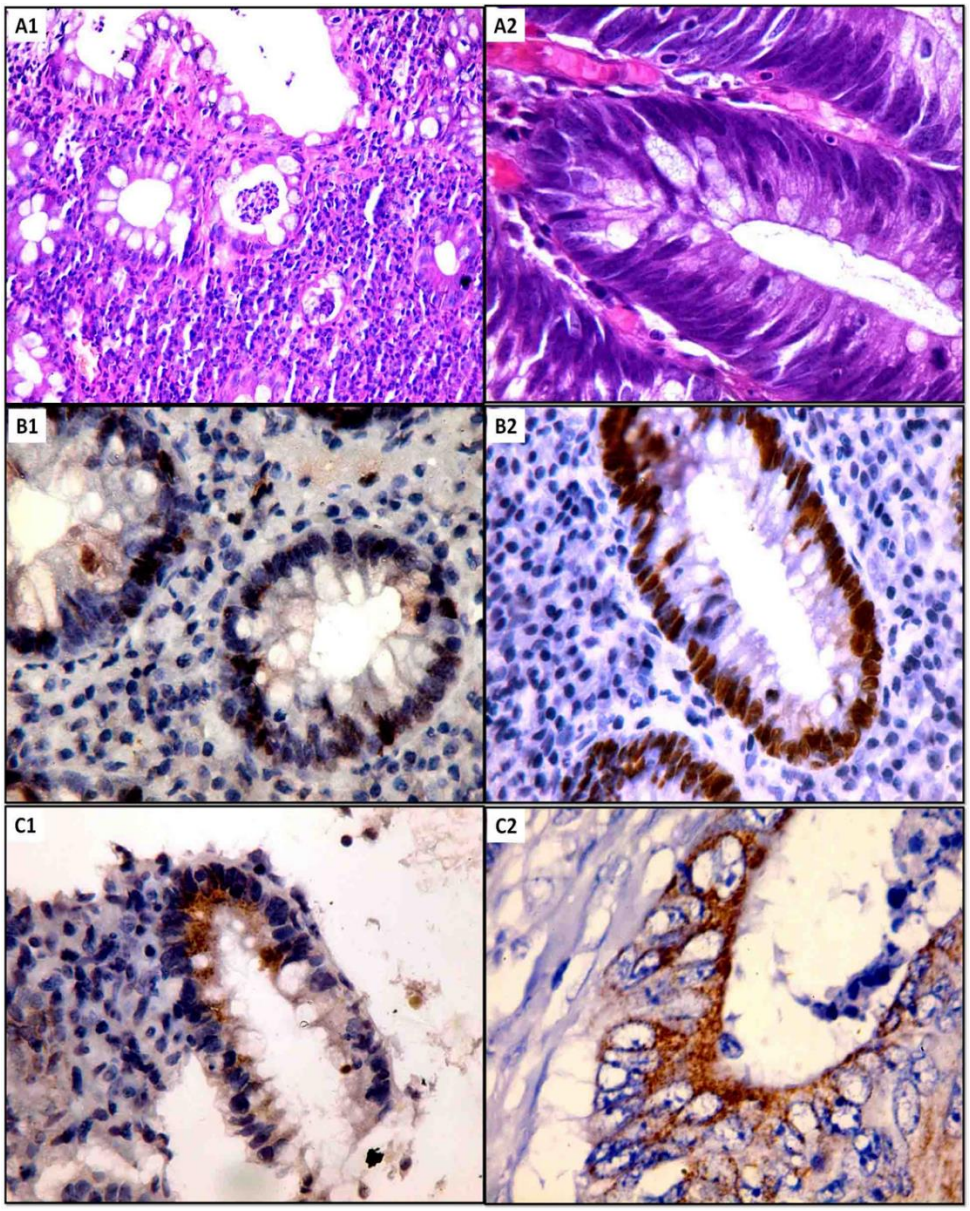

Figure 3. Images (A1, A2) illustrating H&E histological evaluation. A1: Active ulcerative colitis IND showing surface ulceration, intense inflammation, and crypt abscess. Riddell's criteria score was 0 (baseline assessment) (H&E 200X). A2: low-grade dysplasia Riddell's criteria score 2 (follow-up assessment) (H&E 1000X).
Images (B1, B2) illustrating immunohistochemical expression patterns of p53. B1 shows p53 expression in a patient with IND score 2 (baseline assessment) (400X) B2: shows p53 overexpression in a patient with low-grade dysplasia score 3 (follow-up assessment) (400X)
Images (C1, C2) illustrating immunohistochemical expression patterns of AMACR. C1 shows AMACR expression in a patient with IND score 4 (baseline assessment) (400X) B2: shows AMACR overexpression in a patient with high-grade dysplasia score 7 (follow-up assessment) (1000X)

https://dx.doi.org/10.21608/zumj.2020.47756.1983
Volume 28, Issue 4, July 2022(748-761)

DISCUSSION

Does CMV Cause IBD, Complicate IBD, exacerbate IBD, or Is It Just an "Innocent Bystander?"

IBD patients are usually immunosuppressed due to immunosuppressive medications, poor nutrition, and impairment of natural killer function. These factors, as well as CMV tropism for sites of inflammation, leave them at increased risk for active CMV infection and disease. Early studies indicated that CMV may lead to the subsequent development of IBD. CMV colitis has occurred primarily in patients with preexisting UC, with the documented disease for as long as 20–30 years in some cases. Therefore, although CMV infection may cause subsequent development of chronic IBD in some susceptible patients, this does not seem to be the case for most individuals. Another theory was that CMV was an innocent bystander in IBD colitis; this was based on experimental studies that showed that rapidly proliferating cells in granulation tissue are susceptible to CMV infection. Many previous cases, however, have shown that some severe, refractory IBD colitis flares have been associated with documented CMV inclusion bodies, and in some instances a 15% toxic dilation rate, a 62% colectomy rate, and a 44% mortality rate. The current most widely held theory is that CMV infects areas of active IBD and causes colonic injury in most cases. TNF-αand IFN-γ is elevated in IBD patients, and this causes reactivation of latent CMV infection, leading to colonic injury and necrosis. Active CMV infection in these immunosuppressed individuals can lead to severe disease, and CMV itself causes the liberation of proinflammatory cytokines, such as IL-6, that further exacerbate the inflammatory process of IBD colitis.[6]

In our study, we found that CMV reactivation is significantly associated with a colonic injury with overexpression of P53 & AMACR, where we found that there was a significant association between CMV reactivation and CMV-UC with P53&AMACR group whereas it is associated with the highest rate of CMV reactivation 9/22 (40.9%) cases compared to the other group, 1/15 (5.6%) case of reactivation in CMV-UC without P53&AMACR group, ($p<0.001$). In addition to CMV, reactivation is associated with treatment resistance and progression of the disease [22]

The benefit of anti-CMV therapy on the evolution of UC in patients with CMV reactivation

Nakase et al, [20] claim note that' Multiple reports have reported a response to corticosteroid therapy for antiviral care in UC patients with CMV infection. They assume, however, that neither antiviral therapy is required in all UC patients where HCMV is found. The exact method to classify patients with antiviral therapy changing their health has yet to be found. Consequently, UC patients with concomitant HCMV inflammation have no standard clinical protocol.

For this reason, we note in our study that CMV infected UC cases with negative P53&AMACR overexpression had a regressive improvement course such as clinical improvement, histological improvement after antiviral treatment, while CMV infected UC cases with identification of both P53&AMACR had a progressively worsening course.

A lot of case reports, as well as punctual prospective studies, have reported a clinical improvement associated with a reduction of colectomy rate when UC patients with CMV reactivation received ganciclovir [7],

Our analysis investigated 40 CMV-UC patients, 30 of them (75%) have CMV remission and have been clinically and histologically improved following treatment with ganciclovir, while the remaining ten patients showed an increase in p53&AMACR expression and 8 of them showed clinical and histological deterioration, at follow up assessment. This was in line with Pillet, et al, [7]. Found that, from a total of 58 CMV-UC treated patients, 46 (79%) cases showed a clinical improvement and 11 justified colectomies (18%). This was also in keeping with the results found by Roblin et al, [22] who found that 7/16 patients with CMV- UC received ganciclovir; they showed a clinical improvement.

In UC patients with CMV infection, on the other hand, a meta-analysis was conducted recently to determine the impact of antivirals on the colectomy rate. The meta-study involved 15 trials with a total of 333 patients; 43.2% provided antiviral therapy and 56.8% did not. In seven trials, HE and IHC are primarily identified, and in four tissues PCR. There was no difference in colectomy between antiviral and treatment-less patients (OR= 0.92; 95% CI: 0.31-2.76), in the patient's collectivity. [7]

This discrepancy could be attributed to the condition of p53/AMACR expression has not been checked in the previously studied cases,

Mechanism of action of CMV

Infection of host cells with human cytomegalovirus (HCMV) induces cell cycle dysregulation through cellular accumulation of high levels of p53 protein in HCMV-infected cells, but p21 which is (the

indicative marker of p53 transcriptional activity) is markedly decreased. HCMV has two (IE) proteins, IE1-72 and IE2-86, which are promiscuous transactivators that have been implicated in dysregulation events. Both IE1-72 and IE2-86 were able to transactivate the p53 promoter and interact with p53 protein in HCMV-infected cells preventing or disrupting p53 binding to p53-specific DNA sequences and enhancing p53 binding and inducing supershift of this DNA-protein complex resulting in multiple outcomes, such as cellular p53 accumulation, stimulation of cellular DNA synthesis, cell cycle progression and cell cycle arrest, and prevention of program cell death.[8]. Besides, the specific binding of the HCMV mtrII oncoprotein to p53 and the stabilization of p53 protein in mtrII-transformed cells lead to concomitant inhibition of p53-activated transcription and inhibits p53 nuclear localization signals [5][22]

P53

P53 is a tumor suppressor gene, located on chromosome 17p, plays a pivotal role in cell proliferation. The known functions of p53 include its action as a G checkpoint of the cell cycle, which delays the entry of genetically damaged cells into S phase until their damaged DNA can be repaired, otherwise, apoptosis may occur. Histological progression towards cancer occurs in a stepwise fashion in UC (negative -+ indefinite for dysplasia + dysplasia -+ cancer). Foci of high-grade dysplasia or even carcinoma, are often surrounded by regions of mucosa that indefinite for dysplasia (IND) but contain a p53 mutation.[24] p53 mutation is the most promising biomarker of colonic premalignancy and could be used in combination with the histological evaluation to identify patients at high risk of developing colorectal cancer[25] as in UC-associated colonic cancer, P35 mutations were detected in the mucosa that was negative or indefinite for dysplasia (IND) however, the detection of p53 mutation in normal colonic mucosa has not been previously reported][9]. From this point of view, we investigate p53 expression in non-dysplastic mucosa of CMV-UC patients and follow-up for progression and treatment resistance

We found that 39% of cases of CMV-UC patients with non-dysplastic mucosa showed P53 expression, this was away from the results found by Van Schaik et al, [10] and Takaku et al, [9] who found p53 expression in non-dysplastic mucosa of UC patients were 17% and 28 % respectively this may be due to a small number of cases and non-CMV UC cases.

.AMACR

α-methylacyl-CoA-racemase (AMACR), an enzyme that catalyzes the racemization of α-methyl-branched carboxylic coenzyme. Immunohistochemical analysis of AMACR in IBD-associated colorectal cancer could serve as a useful adjunct to histological analysis in the evaluation of dysplastic lesions that are difficult to classify by histological observations. AMACR levels were increased in biopsy samples positive for dysplasia (both LGD and HGD) compared with non-dysplastic samples. [25]

In our study, we found that twenty-three cases, 37% at baseline assessment, whereas the mucosa shows active colitis, showed AMACR expression. This was in agreement with Van Schaik et al, [10] who found that five of twelve cases (42%) of UC patients without dysplasia showed expression of AMACR. Away of our results was Dorer, et al, [26] who found AMACR expression in non-dysplastic and indefinite dysplastic mucosa of UC cases was one of seven cases (14%). This difference may be due to a small number of cases and non-CMV infected UC cases.

P53 & AMACR Coexpression

This study mainly investigated the role of combined expression of P53 and AMACAR in predicting the treatment-resistant risk of dysplastic or neoplastic progression and CMV reactivation in patients with CMV infected ulcerative colitis We found that 36.4% of CMV-UC with p53&AMACR group are significantly developed clinical progression (Hanauer's index) and histological progression including both inflammatory index (Riley's score) and dysplastic index (Riddell's score) and 40.9% of them showed CMV reactivation after following up assessment. In comparison to CMV-UC patients without P53&AMACR coexpression, only 5.6% of patients developed clinical progression, 11.1% developed histological progression, and 5.6% CMV reactivation after follow-up assessment. While in Non CMV-UC with P53 & AMACR, the results were 11.1% of patients developed clinical progression, and 22.2% developed histological progression. This is partially in agreement with Van Schaik et al, [10] who found that 86% of cases with P53/AMACR coexpression developed neoplastic progression in 19 months.

CONCLUSION

P53/AMACR co-expression is an early indicator of dysplastic progression, treatment resistance, and clinical deterioration. Patients with UC should have a regular examination for CMV infection and early CMV treatment before mutations of p53 and

AMACR are overexpressed, as their presence reduces the chances of recovery and accelerates dysplastic progression.

Conflicts of interest: None
Financial disclosure: None

REFERENCES

1. Yoon JY, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Correlation of C-reactive protein levels and erythrocyte sedimentation rates with endoscopic activity indices in patients with ulcerative colitis. Dig Dis Sci. 2014;59(4):829-37.
2. Fumery M, Dulai PS, Gupta S, Prokop LJ, Ramamoorthy S, Sandborn WJ, et al. Incidence, risk factors, and outcomes of colorectal cancer in patients with ulcerative colitis with low-grade dysplasia: a systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2017;15(5):665-74. e5.
3. Williams JG, Alam MF, Alrubaiy L, Arnott I, Clement C, Cohen D, et al. Infliximab versus ciclosporin for steroid-resistant acute severe ulcerative colitis (CONSTRUCT): a mixed methods, open-label, pragmatic randomised trial. The lancet Gastroenterology & hepatology. 2016;1(1):15-24.
4. Lv R, Qiao W, Wu Z, Wang Y, Dai S, Liu Q, et al. Tumor necrosis factor alpha blocking agents as treatment for ulcerative colitis intolerant or refractory to conventional medical therapy: a meta-analysis. PLoS One. 2014;9(1):e86692.
5. Roblin X, Pillet S, Oussalah A, Berthelot P, Del Tedesco E, Phelip J-M, et al. Cytomegalovirus load in inflamed intestinal tissue is predictive of resistance to immunosuppressive therapy in ulcerative colitis. Am J Gastroenterol. 2011;106(11):2001-8.
6. Kandiel A, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. Am J Gastroenterol. 2006;101(12):2857-65.
7. Pillet S, Pozzetto B, Roblin X. Cytomegalovirus and ulcerative colitis: Place of antiviral therapy. World J Gastroenterol. 2016;22(6):2030.
8. Hwang E-S, Zhang Z, Cai H, Huang DY, Huong S-M, Cha C-Y, et al. Human cytomegalovirus IE1-72 protein interacts with p53 and inhibits p53-dependent transactivation by a mechanism different from that of IE2-86 protein. J Virol. 2009;83(23):12388-98.
9. Takaku H, Ajioka Y, Watanabe H, Hashidate H, Yamada S, Yokoyama J, et al. Mutations of p53 in Morphologically Non-neoplastic Mucosa of Long-standing Ulcerative Colitis. Jpn J Cancer Res. 2001;92(2):119-26.
10. Van Schaik FD, Oldenburg B, Offerhaus JG, Schipper ME, Vleggaar FP, Siersema PD, et al. Role of immunohistochemical markers in predicting progression of dysplasia to advanced neoplasia in patients with ulcerative colitis. Inflamm Bowel Dis. 2012;18(3):480-8.
11. Nikolaus S, Schreiber S. Diagnostics of inflammatory bowel disease. Gastroenterology. 2007;133(5):1670-89.
12. Kim YS, Kim Y-H, Kim JS, Cheon JH, Ye BD, Jung S-A, et al. The prevalence and efficacy of ganciclovir on steroid-refractory ulcerative colitis with cytomegalovirus infection: a prospective multicenter study. J Clin Gastroenterol. 2012;46(1):51-6.
13. Rosenberg L, Nanda KS, Zenlea T, Gifford A, Lawlor GO, Falchuk KR, et al. Histologic markers of inflammation in patients with ulcerative colitis in clinical remission. Clin Gastroenterol Hepatol. 2013;11(8):991-6.
14. Lemmens B, Arijs I, Van Assche G, Sagaert X, Geboes K, Ferrante M, et al. Correlation between the endoscopic and histologic score in assessing the activity of ulcerative colitis. Inflamm Bowel Dis. 2013;19(6):1194-201.
15. Riddell RH, Goldman H, Ransohoff DF, Appelman HD, Fenoglio CM, Haggitt RC, et al. Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum Pathol. 1983;14(11):931-68.
16. Kambham N, Vij R, Cartwright CA, Longacre T. Cytomegalovirus infection in steroid-refractory ulcerative colitis: a case-control study. The American journal of surgical pathology. 2004;28(3):365-73.
17. Beaugerie L, Cywiner-Golenzer C, Monfort L, Girard P-M, Carbonnel F, Ngô Y, et al. Definition and diagnosis of cytomegalovirus colitis in patients infected by human immunodeficiency virus. JAIDS Journal of Acquired Immune Deficiency Syndromes. 1997;14(5):423-9.
18. Huang W, Zhao J, Li L, Huang Y, Yang X, Wang J, et al. a-Methylacyl coenzyme A racemase is highly expressed in the intestinal-type adenocarcinoma and high-grade dysplasia lesions of the stomach. Histol Histopathol. 2008.
19. Kim YS, Kim Y-H, Kim JS, Jeong SY, Park SJ, Cheon JH, et al. Long-term outcomes of cytomegalovirus reactivation in patients with moderate to severe ulcerative colitis: a multicenter study. Gut and liver. 2014;8(6):643.
20. Nakase H, Honzawa Y, Toyonaga T, Yamada S, Minami N, Yoshino T, et al. Diagnosis and treatment of ulcerative colitis with cytomegalovirus infection: importance of controlling mucosal inflammation to prevent cytomegalovirus reactivation. Intestinal research. 2014;12(1):5.
21. Yoshino T, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, et al. Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies. Inflamm Bowel Dis. 2007;13(12):1516-21.
22. Muralidhar S, Doniger J, Mendelson E, Araujo JC, Kashanchi F, Azumi N, et al. Human cytomegalovirus mtrII oncoprotein binds to p53 and down-regulates p53-activated transcription. J Virol. 1996;70(12):8691-700.
23. Wang J, Belcher JD, Marker PH, Wilcken DE, Vercellotti GM, Wang XL. Cytomegalovirus inhibits p53 nuclear localization signal function. J Mol Med. 2001;78(11):642-7.
24. Brentnall TA, Crispin DA, Rabinovitch PS, Haggitt RC, Rubin CE, Stevens AC, et al. Mutations in the p53 gene:

an early marker of neoplastic progression in ulcerative colitis. Gastroenterology. 1994;107(2):369-78.
25. Thorsteinsdottir S, Gudjonsson T, Nielsen OH, Vainer B, Seidelin JB. Pathogenesis and biomarkers of carcinogenesis in ulcerative colitis. Nature reviews Gastroenterology & hepatology. 2011;8(7):395-404.
26. Dorer R, Odze RD. AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, ulcerative colitis, and Crohn's disease. The American journal of surgical pathology. 2006;30(7):

Alabiad, M., Fakhr, A., Mohamed, A., Gomaa, A., Shalaby, A., Nosery, Y., Gobran, M. P53&AMCR over-expression accelerate dysplastic progression in CMV ulcerative colitis patients (a comparative study of CMV and Non-CMV ulcerative colitis). *Zagazig University Medical Journal*, 2022; (748-761): -. doi: 10.21608/zumj.2020.47756.1983