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The Role of Manganese Superoxide Dismutase as a Prognostic Marker in Egyptian Patients with Ulcerative Colitis

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Abstract: Background: Ulcerative colitis (UC) is a disease characterized by remitting and relapsing inflammation of the colon. Clinical, endoscopic, radiological and histopathological findings play a major role in the diagnosis and assessment of inflammatory degree and disease severity in UC but it is hard to predict the clinical outcome for a patient. We aimed to evaluate the role of Manganese Superoxide Dismutase as a prognostic marker in Egyptian Patients with Ulcerative Colitis by detection of its relation to severity of illness by colonoscopy findings, inflammatory status and patient outcome**. Methods:** We analyzed MnSOD expression immunohistochemically in 80 formalin-fixed and paraffin-embedded specimens from 80 patients, including 26 ulcerative colitis patients with chronic continuous (CC)/symptoms remaining (SR) type, 24 ulcerative colitis patients with remission relapse (RR)/exacerbation (E) type and 30 subjects as control, who underwent colonoscopy examinations and biopsy. MnSOD expression was observed in lamina propria. **Results:** We found the expression pattern of MnSOD in lamina propria showed significant difference among the studied groups (P value <0.001) in which, it is more positive in chronic continuous (CC) patients. It was more frequently found in cases of CC and SR type and showed significant positive correlation with disease severity. **Conclusion**: Immunohistochemical evaluation for MnSOD expression may be helpful for early detection of the degree of severity, activity and prediction of progression of UC.
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1. Introduction

The inflammatory bowel diseases (IBD) are lifelong, relapsing-remitting diseases affecting physical, psychological, familial, and social aspects of life [1]. The highest prevalence of IBD was reported in Europe and North America while the incidence is increasing in newly industrialized countries (in Africa, Asia and South America) [2].

Ulcerative colitis (UC) is a form of Inflammatory bowel disease (IBD) characterized by damage of the large bowel mucosa. The most broadly acknowledged theory of IBD pathogenesis is that the mucosal immune system mounts an aberrant response toward luminal antigens, for example, dietary factors and additionally commensal bacteria in genetically susceptible individual [3,4]

Ulcerative colitis course highly varies from localized to extensive colonic affection and from mild symptoms for prolonged periods of remission [5] to severe life-threatening presentations that can lead to hospitalization, colectomy, and death [6].

The assessment of disease activity in IBD patients can be achieved utilizing clinical disease activity indices, endoscopic indices, serum markers, and fecal markers. These indices obtain an indirect estimation of disease activity, but may not predict accurately the inflammatory activity which is detected in endoscopic and histological examinations [7].

The pathological findings from the mucosal biopsy were late proposed as a method for assessment of degree of inflammatory activity and are presently practiced. In spite of the fact that this is useful for evaluation of the degree of inflammatory activity at one time point; it is hard to predict the clinical outcome for a patient. So the emerging of a new marker specific for mucosal inflammation would be helpful for upgrading the accuracy of monitoring disease severity and activity [8].

Oxidative stress has been involved in numerous human diseases, including inflammatory bowel diseases (IBD). Chronic intestinal inflammation has been found to be associated with overrun of both reactive nitrogen and reactive oxygenspecies (RNS and ROS) resulting in nitrosative and oxidative stress, respectively [9].

Superoxide dismutases (SODs) are considered as the primary line of defenses against the harmful effects of ROS production by catalyzing the dismutation of superoxide radical into H2O2, which is removed by catalase or glutathione peroxidase. There are three mammalian SOD proteins; CuZnSOD (SOD1), which resides in the cytosol, nucleus, lysosomes and peroxisomes, ECSOD (SOD3), dwells extracellular fluids and MnSOD (SOD2), which resides in the mitochondria. The majority of superoxide anions have been converted into less reactiveH2O2[10, 11, 12] on site by MnSOD so it is vital for cell survival [13, 14].

Induction of MnSOD is defensive against radical-mediated damage similarly as against interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-a) cytotoxicity [15].

Regulation of MnSOD may play role in the protection against cytokine toxicity and mitochondrial oxygen radical generation. TNF - a, IL - l, similar to bacterial endotoxin, regulateMnSOD expression in the intestinal epithelial cell [16].

This study aimed to evaluate the role of Manganese Superoxide Dismutase as a prognostic marker in Egyptian Patients with Ulcerative Colitis by detection of its relation to severity of illness by colonoscopy findings, inflammatory status and patient outcome.

2. Methods

This is a cross sectional study performed on 80 subjectsfrom the outpatient clinics and inpatients of Tropical Medicine and Infectious Diseases Department in Tanta University Hospitals within one year period between 2016 till 2017. The study protocol was approved by Institutional ethical committee. Written informed consent was obtained from every participant before the beginning of the study.

Demographic data, clinical information, symptoms, treatment, and endoscopic grading were recorded and multiple mucosal biopsies were obtained from colonic mucosa from every subject under complete aseptic precautions. The subjects of the study were classified into two groups**: Group I**: Consists of 50ulcerative colitis patients who were classified according to inflammation status and patient outcome into **Group IA:** included 26ulcerative colitis patients with chronic continuous (CC)/symptoms remaining (SR) type **Group IB:** included 24ulcerative colitis patients with remission relapse (RR)/exacerbation (E) type. **Group II**: included 30 subjects as control group, who performed the lower intestinal endoscopy for purposes other than ulcerative colitis.

Inclusion criteria: Patients with colonscopic and histopathologic findings of Ulcerative colitis and Ulcerative colitis patients under surveillance colonscopic examination were included in the study. Exclusion criteria: patients who refused to participate in the study and patients with Pregnancy, malignancy, liver failure, heart failure or renal failure were excluded.

Data collection

Informed consent was taken from every patient after explaining the whole procedure. Demographic data were collected. History taking, clinical examination, laboratory investigations including complete blood picture, liver function tests, ESR, and radiological investigations including Plain X – ray on abdomen and pelvis were done. Colonoscopy was done to all groups under sedation and endoscopic findings were recorded. Endoscopic activity of ulcerative colitis evaluation was based on the Mayo score flexible proctosigmoidoscopy assessment from 0 to 3 and multiple biopsies were taken for histopathological examination [17].

Haematoxylin and Eosin (H & E) staining

All samples were archived formalin-fixed and paraffin embedded tissue, which were cut into 3 to 5 mm sections and fixed by light heating to be stained by haematoxylin-eosin stain then examined under light microscopy using magnification powers of 40, 80, and 120. The H & E stained specimens were histologically classified according to Geboes classificationfrom0-5 grade [18].

Immunohistochemical staining:

Sections (3-4u) were cut from formalin fixed paraffin embedded blocks on positive charged slides then dewaxed in fresh xylene bath and incubated at room temperature for 5 minutes twice then immersed in 3% hydrogen peroxide in methanol for 30 minutes and washed by PBS for 5 minutes. Sections were immersed in 10ml mol/L Citrate buffer (PH 6.0) for 10 minutes at 100°c in a microwave. Two to three drops of nonspecific blocking reagent and two or three drops of MnSOD were placed on each slide then washed for 5 minutes in PBS. Two to three drops of the secondary biotinylated Ab were placed on each slide and incubated in the humidity chamber at room temperature for 30 minutes then washed for 5 minutes in PBS. Two to three drops of streptavidin enzyme label were placed on each slide and incubated for 30 minutes at room temperature in the humidity chamber then washed for 5 minutes in PBS. Diamino-Benzedine (DAB) chromagen was prepared by addition of one drop of DAB chromagen per one ml of buffered substrate then several drops of this reagent were placed on each slide and washed in tap water Finally, stained with Mayer`s haematoxylin for one minute and washed in tap water. Mnsod reactivity was evaluated according to staining intensity graded as 0(negative) demonstrated negative staining, 1(weak)

represented less than 10%, 2(moderate) represented 10–50%, and 3(strong) represented more than 50%.

Statistical Analysis:

Data were collected, reviewed and fed to the computer where statistical analysis was done using statistical package by SPSS® V.19. The following basic statistics were used: Mean value, Standard Deviation [SD] The chi-square test or Fisher's exact test was used to analyze the categorical variables and a P value of, 0.05 was considered to be statistically significant.

3. Results

A total number of 80 patients were enrolled in the study 50 patients with ulcerative colitis who were classified according to inflammation status and patient outcome into **Group IA:**26 ulcerative colitis patients with chronic continuous (CC)/symptoms remaining (SR) type **Group IB:** included 24 ulcerative colitis patients with remission relapse (RR)/exacerbation (E) type. And **Group** II: 30 peoples as control. Demographic data was demonstrated in the table (1) in which age and gender were not significantly different among the studied groups (p >0.05). As regards clinic-laboratory data of both UC patients' groups, there were significant differences in blood in stool, number of motion/day, pulse rate, albumin, Hb., ESR 1st and ESR 2nd (p< 0.05). (Table 1).

The histopathological scoring by H & E using Geboes classification show a significant difference between both UC patients' groups (P value <0.001) as chronic continuous (CC) group specimens had arranged from 3 – 5, while remission relapse (RR) group specimens had arranged from 1 – 3.

Classification of UC cases according to Mayo score was as follows: among chronic continuous (CC) patients, two specimens (7.69%) were mild; ten (38.46%) were moderate; 14 (53.85%) were sever However remission relapse (RR) patients, ten specimens (41.67%) were mild; ten (41.67%) were moderate; four (16.67%) were sever. As for all UC patients, the treatment with medication was provided. The salicylazosulphapyridine as nonsteroid treatment was given for (58.33%) remission relapse (RR) and (7.69%) chronic continuous (CC) while, the combination therapy with the steroid (prednisolone) was given for (41.67%) remission relapse (RR) and (92.31%) chronic continuous (CC).

In Table (2), the expression pattern of MnSOD in lamina propria showed significant difference among the studied groups (P value <0.001) in which, it is more positive in chronic continuous (CC) patients as shown in Fig. 1. This expression pattern was more frequently found in cases of CC and SR type (Table 3).

The expression pattern of MnSOD in lamina propria showed significant positive correlation with H & E Geboes score, Number of motion/day, Pulse rate, S albumin, HB%, ESR and blood in stool (Table 4).

Table 1. Comparison between demographic data and clinic-laboratory data of both UC patients' groups

Group	Chronic continuous (CC) /Symptoms remaining (SR) (n=26)	Remission relapse (RR) /Excerbation (E) (n=24)	P – value
Age (mean + SD)	33.00 ± 11.43	36.83 ± 9.65	0.096
Gender Male / Female	20 / 6	14 / 10	0.298
Blood in stool	5.078± 1.23	3.50± 1.22	<0.001*
Duration of disease in years	3.46± 1.63	3.08± 1.21	0.475
Number of motion/day	7.69± 3.97	4.75± 1.68	0.001*
Pulse rate	89.23± 11.09	81.33± 6.84	0.003*
Albumin (gm/dl)	3.35± 0.35	3.83± 0.46	0.008*
Hb. (gm/dl)	32.39± 2.70	40.42± 4.36	<0.001*
ESR 1st	1.20 ± 0.11	1.36 ± 0.34	<0.001*
ESR 2nd	105.07 ± 87.65	25.59 ± 35.74	<0.001*

*Significant.

Table 2. Comparison between MnSOD expression in lamina propria of the three groups

Group	Mnsod in endothelium		
	Range	Mean ± SD	P – value
Chronic continuous	4 - 6	5.15 ± 0.78	<0.001*
Remission relapse	1 - 4	2.25 ± 0.94	
Control	0 - 3	1.20 ± 1.09	

Significant TUKEY'S Test (I & II), (I & III) and (II & III)= <0.001

Table 3. Correlation between expression of MnSOD in lamina propria and (two typing clinical course & Mayo score) among UC patients' groups

		MnSOD in endothelium		
		N	Mean ± SD	P – value
Mayo score	Mild	12	0.94 ± 2.17	<0.001*
	Moderate	20	3.40 ± 1.46	
	Severe	18	5.22 ± 1.06	
Inflammatory status	Chronic continuous	26	5.15 ± 0.78	<0.001*
	Remission relapse	24	2.25 ± 0.94	
patient outcome	Symptoms remaining	26	5.00 ± 0.94	<0.001*
	Exacerbation	24	2.18±0.96	

*Significant

Table 4. Correlation between expression of MnSOD in lamina propria and clinicopathological factors

	MnSOD in endothelium	
	r	P – value
H & E Geboes score	0.915	<0.001*
Number of motion/day	0.415	0.003*
Pulse rate	0.402	0.004*
S albumin	0.307	0.030*
HB%	0.783	<0.001*
ESR 1st	0.318	0.024*
ESR 2nd	0.289	0.042*
Blood in stool	0.613	<0.001*

*Significant

4. Discussion

IBD is related to an imbalance between ROS and antioxidant activity that causes oxidative stress due to either overproduction of ROS and reduced antioxidant activity [19]. This condition is potentially hazardous since it can alter the inflammatory response and lead to changes in proteins and lipids, DNA damage, apoptosis or cancer cell transformation [20], [21] and [22].

The manganese superoxide dismutase (MnSOD) situated in the mitochondria effectively converts superoxide to the less reactive hydrogen peroxide (HO), which can further break down by other enzymatic and non - enzymatic antioxidants into water and dioxygen [23]. MnSOD assumes an essential role in the maintenance of gastrointestinal tract homeostasis [24].

Fagerberg *et al* [25] stated that hemoglobin, hematocrit, to talleucocytic count, platelets and ESR are routinely used as inflammatory markers in blood when UC is suspected, however, these markers correlate poorly with endoscopic and histological examination. Although ESR provides patients with suspected UC with a helpful screening test, it is not completely reliable [26]. These clinical indices are not IBD specific and indirectly measure the activity and severity of the disease and may not predict inflammatory activity accurately [8].

Colonoscopy and mucosal biopsy are considered to be the most accurate and objective colorectal inflammation measure and the gold standard for UC diagnosis [27,28]. Mayo score is considered the best known tool for disease activity [29].

In this work we studied the expression of MnSOD comparing the results with group of UC patients with CC, SR course and patients with RR, E course and control group. This was supported by the study done by **Ikumoto *et al*** [8] that concluded that the expression of MnSOD was closely correlated with the type of disease and patients with a mild degree expressing MnSOD tended to have symptom stabilization. **Sakthivel and Guruvayoorappan** [30] found that the levels of SOD motivated by intrarectal acetic acid increase in UC. The increase in SOD and other antioxidants can be clarified mostly by the presence of inflammation and OS in IBD and this SOD increase would protect the tissue against oxidative damage. The study done by **Rana and Sharma** [31] to evaluate the Role of oxidative stress & antioxidant defense in ulcerative colitis patients from north India demonstrated that SOD were found to be significantly increased in UC patients compared to control.

In concordance with these were the results found by **Piecuch *et al*** [32] who concluded that in both premalignant and malignant colorectal lesions, the expression of MnSOD increased.

We found positive correlation between MnSOD over expression in lamina propria and degree of inflammation and severity of illness as its expression was severe in chronic continuous, symptoms remaining UC patients and mild in relapse remission, exacerbation UC patients.

This can be explained by; long duration of UC denoting long and severe exposure to inflammation which may lead to over expression of MnSOD as shown in the previous studies done by **Ikumoto *et al*** [8] may be the mechanism by which chronic inflammation lead to oxidative stress. MnSOD immunoexpression is companied by clinicopathological findings in UC.

In our study, the correlation between severe and active inflammation and long standing chronic UC based on a statistically relevant analysis of the immunohistochemical marker MnSOD. Adding this immunohistochemical marker to routine histological assessment may improve the accuracy of early detection of the degree of severity and activity also correlate directly with the progression of UC.

So we finally concluded that MnSOD can be used for early evaluation of the prognosis of ulcerative colitis patients.

Assessment of MnSOD immunostaining should be combined with routine histological evaluation in longstanding UC to improve the accuracy of early detection of the degree of severity and activity also correlate directly with the progression of UC. Our findings warrant further exploration and validation within large multicenter studies.

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Author contributions:

All authors were involved in designed the study, designed the data collection, instruments, coordinated, supervised data collection and analysis, critical review, editing, revision, and approval of the final manuscript.

Bullet points of the study:

What is already known?

The assessment of disease activity in IBD patients can be achieved utilizing clinical disease activity indices, endoscopic indices, serum markers, and fecal markers.

What is new in this study?

The role of Manganese Superoxide Dismutase as a prognostic marker in Egyptian Patients with Ulcerative Colitis by detection of its relation to severity of illness, inflammatory status.

What are the future clinical and research implications of the study findings?

Assessment of MnSOD immunostaining should be combined with routine histological evaluation in longstanding UC to improve the accuracy of early detection of the degree of severity and activity.

Text:

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