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PLATELET LEUKOCYTE AGGREGATES AS A PRO-THROMBOTIC RISK FACTOR IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE

By

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ABSTRACT

Background: Patients with inflammatory bowel disease (IBD) are at an increased risk for venous thromboembolism (VTE). VTE events carry significant morbidity and mortality, and have been associated with worse outcomes in patients with IBD. Studies have suggested that the hypercoagulable nature of the disease stems from a complex interplay of systems that include the coagulation cascade, endothelium, immune system, and platelets. Additionally, clinical factors that increase the likelihood of a VTE event among IBD patients include pregnancy, active disease, more extensive disease, hospitalization, and IBD-related surgeries.

Objective: To assess the platelet-leukocyte aggregation in patients with IBD and its relation to disease activity.

Patients and Methods: This was a prospective cohort study conducted on 40 IBD Patients and 20 control cases who were admitted to Al-Hussein University Hospital, and some selected from outpatient clinic, during the period from 15th of April, 2020 to 31st of March, 2021. All participants underwent to clinical examination, laboratory findings, colonoscopy, histopathology and flowcytometric findings were recorded from patients.

Results: There was a statistical significant difference (p-value < 0.001) between studied groups as regard hemoglobin level, white blood cells and platelets count. Statistical significant difference (p-value < 0.05) was found between studied groups as regard erythrocytic sedimentation rate and statistical significant difference (p-value < 0.001) between studied groups as regard C reactive protein and Fecal calprotectin. Also, there was a statistical significant difference (p-value < 0.001) between studied groups as regard ulcer, ileitis and edematous mucosa. Statistical significant difference (p-value < 0.001) was found between studied groups as regard platelet leukocyte aggregates.

Conclusion: In patients with active inflammatory bowel disease (IBD), there was a significant high level of platelet leukocyte aggregates (PLAs), which might be explanation phenomena of increased risk for thrombosis.

Keywords: IBD, PLAs, VTE.

INTRODUCTION

Venous thromboembolism (VTE) is one of the well-studied extra intestinal manifestations of IBD, which includes deep vein thrombosis and pulmonary embolism. The higher risk of VTE in IBD patients is related to multiple factors. One major factor would be systemic inflammation, which causes a

hypercoagulable state due to the activation of the coagulation cascade and platelet aggregation *(Saibeni et al., 2010)*. IBD patients were found to have a 2- to 3-fold risk of VTE compared to non-IBD patients; increasing up to 8-fold during an IBD flares *(Wallaert et al., 2012)*. VTE in IBD patients is associated with higher mortality, morbidity and in-hospital cost compared to non-IBD patients. Hospital stay and hospital charges were double in IBD patients who developed VTE compared to non-VTE IBD patients *(Yuhara et al., 2013)*. Inflammatory bowel disease (IBD) is a chronic disease that affects the gastrointestinal tract and also has extra intestinal manifestations. IBD has 2 major disease entities: crohn's disease (CD) and ulcerative colitis (UC) *(Ananthakrishnan et al., 2018)*.

PATIENTS AND METHODS

This prospective cohort study has been carried out at Al-Hussein University Hospital over a period of eleven months, from April-2020 to March 2021, and conducted on 40 IBD Patients and 20 control cases. Before starting the study, approval from the Ethics Committee, Faculty of Medicine, Al-Azhar University, Cairo, Egypt, was obtained. Additionally, an informed consent was obtained from every subject before recruitment for use of their medical reports. **The inclusion criteria included,** age range from 20-50 years, IBD patients confirmed with colonoscopy and histopathology. **There were exclusion criteria that** included patients with history suggesting hypercoagulable state, deep venous thrombosis (DVT). **All patients were subjected to detailed medical history** for name, age, sex, alcohol, occupational and drug history and history of other comorbid conditions), **Laboratory investigations included** complete blood count (CBC), liver function tests (alanine amino transferase (ALT), aspartate amino transferase (AST) and serum albumin), renal function tests (serum creatinine and blood urea), ESR, CRP, fecal calprotectin. Assesment of disease severity according to Montreal classification in ulcerative colitis and Chron´s disease activity index, colonoscopy and histopathology. Platelet leukocyte aggregates (by detecting cluster of differentiation (CD) 41 as a pan platelet marker and CD45 as a pan leukocyte marker by flowcytometry).

Statistical analyses: Data were analyzed using Statistical Package for the Social Science (SPSS) version 24. Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- **Independent-samples t-test** of significance was used when comparing between two means.
- **Mann Whitney U test** was used when comparing between two means (for abnormal distributed data).
- **Chi-square test** was used when comparing between non-parametric data.
- **A one-way analysis of variance (ANOVA)** was used on comparing between more than two means.
- **Post Hoc test** was used for multiple comparisons between different variables.

RESULTS

Regarding description of demographic data, the median age of studied patients was 33.5 (28.3 – 40) and the median age of studied control cases was 33 (23.8 - 39.8). There were 18 diseased males (45%) and 22 diseased females (55%) and 12 control males (60%) and 8 control females (40%) in all studied patients. There were 4 diabetic (10%) and 5 hypertensive (13%) in studied patients and 1 diabetic (5%) and 2 hypertensive (10%) in control cases. There was no statistical significant difference (p-value > 0.05) between studied groups as regard age and sex, and no statistical significant difference (p-value > 0.05) between studied groups as regard to chronic diseases as diabetes mellitus (DM) and hypertension (HTN) **(Table 1)**.

Table (1): Comparison between studied groups as regard demographic data

Variables \ Groups		Group A (N = 40)		Group B (N = 20)		P-value
Age (years)	Median	33.5		33		0.649
	IQR	28.3 - 40		23.8 - 39.8		
Sex	Male	18	45%	12	60%	0.273
	Female	22	55%	8	40%	
DM	No	36	90%	19	95%	0.509
	Yes	4	10%	1	5%	
HTN	No	35	88%	18	90%	0.776
	Yes	5	13%	2	10%	

According to laboratory profiles, results showed that, the mean of all studied patients was 10 ± 1.6 (g/dl) regarding Hb level and the mean of all studied control cases was 12.5 ± 1.7 (g/dl). Also, results showed that WBCs, PLTs, ESR, CRP, fecal calprotectin, the median of all studied patients was 10.4 (8.7 - 12.9), 440 (362 - 493.8), 31 (18 – 50), 12 (6 – 15), 200 (120.5 - 357.5), respectively. Also, results showed that WBCs, PLTs, ESR, CRP, fecal calprotectin, the median of all studied control cases was 7.3 (5.5 - 8.9), 313.5 (235.8 - 345.8), 20 (15.3 - 30.8), 6 (4.25 - 8.75), 24.5 (15.3 - 35.3), respectively. There was a statistical significant difference (p-value < 0.001) between studied groups as regard Hb level, WBCs and PLTs count. Also there was a statistically significant difference (p-value < 0.05) between studied groups as regard ESR, and a statistical significant difference (p-value < 0.001) between studied groups as regard CRP and fecal calprotectin **(Table 2)**.

Table (2): Comparison between studied groups as regard to laboratory profile

Variables \ Groups		Group A (N = 40)	Group B (N = 20)	P-value
Hb (g/dl)	**Mean**	10.0	12.5	**< 0.001**
	±SD	1.6	1.7	
WBCs (x 10^3/mm^3)	**Median**	10.4	7.3	**< 0.001**
	IQR	8.7 - 12.9	5.5 - 8.9	
PLTs (x 10^3/mm^3)	**Median**	440	313.5	**< 0.001**
	IQR	362 - 493.8	235.8 - 345.8	
ESR (mm/h)	**Median**	31	20	**< 0.002**
	IQR	18 - 50	15.3 - 30.8	
CRP (mg/L)	**Median**	12	6	**< 0.001**
	IQR	6 - 15	4.25 - 8.75	
F.C.P	**Median**	200	24.5	**< 0.001**
	IQR	120.5 - 357.5	15.3 - 35.3	

There was a statistical significant difference (p-value < 0.001) between studied groups as regard ulcer, ileitis and edematous mucosa. Also, there was no statistical significant difference (p-value > 0.05) between studied groups as regard mass, polyp and stricture **(Table 3)**.

Table (3): Comparison between studied groups as regard colonoscopy

Variables \ Groups		Group A(N = 40)		Group B(N = 12)		P-value
Ileitis	**No**	12	30%	12	100%	**< 0.001**
	Yes	28	70%	0	0%	
Ulcer	**No**	9	22.5%	12	100%	**< 0.001**
	Yes	31	77.5%	0	0%	
Mass	**No**	39	97.5%	12	100%	0.51
	Yes	1	2.5%	0	0%	
Polyp	**No**	35	87.5%	12	100%	0.327
	Yes	5	12.5%	0	0%	
Edematus mucosa	**No**	14	35%	12	100%	**< 0.001**
	Yes	26	65%	0	0%	
Stricture	**No**	35	87.5%	12	100%	0.327
	Yes	5	12.5%	0	0%	

Regarding platelet leukocyte aggregates, the mean of all patients was 3649±267.2, and the mean of all control cases was 519±43.1. There was a statistical significant difference between studied groups as regard PLAs **(Table 4)**.

Table (4): Comparison between studied groups as regard platelet leukocyte aggregates (PLAs)

Variables \ Groups		Group A (N = 40)	Group B (N = 20)	P-value
PLAs	**Mean**	3649.0	519.0	**< 0.001**
	±SD	267.2	43.1	

There was a statistical significant difference (p-value < 0.001) of platelet leukocyte aggregates (PLAs) as regard disease activity in group A (Activity assessed according to montreal classification in ulcerative colitis and Crohn's disease activity index in C.D) **(Table 5)**.

Table (5): Comparison of platelet leukocyte aggregates (PLAs) as regard disease activity in patients (group A)

variables \ Activity in group A		Mild (N=40)	Moderate (N=40)	Severe (N = 40)	C.R (N = 20)	P-value
PLAs	**Mean**	3500.5	3673.4	3989.8	3422.0	**< 0.001**
	±SD	258.2	179.9	39.5	133.9	

There was a statistically significant difference between mild active and moderate active patients of group A. Statistical significant difference between mild active and severe active patients of group A. No statistical significant difference between mild active and clinical remission patients of group A. Statistically significant difference between moderate active and severe active patients of group A. Statistically significant difference between moderate active and clinical remission patients of group A. Statistical significant difference between severe active and clinical remission patients of group A **(Table 6)**

Table (6): Post-Hoc test for multiple comparisons between different activity categories as regard PLAs

		LSD	p-value
Mild	**Moderate**	-172.9	0.024
	Severe	-489.3	< 0.001
	Clinical remission	78.5	0.438
Moderate	**Mild**	172.9	0.024
	Severe	-316.4	0.001
	Clinical remission	251.4	0.016
Severe	**Mild**	489.3	< 0.001
	Moderate	316.4	0.001
	Clinical remission	567.8	< 0.001
Clinical remission	**Mild**	-78.5	0.438
	Moderate	-251.4	0.016
	Severe	-567.8	< 0.001

DISCUSSION

The results of current study showed a statistical significant difference between studied groups as regard Hb level, WBCs and Plt count. Also, results showed statistical significant difference between studied groups as regard ESR and statistical significant difference between studied groups as regard CRP and fecal calprotectin. In support to these results, *Larsen et al. (2010)* found that the thrombocytosis in colitis is also accompanied by significant leukocytosis which suggests that the responses may reflect enhanced hematopoiesis. Also, these results ran with *Iskandar and Ciorba (2012)* who found that WBCs may be an indicator of an exacerbation of inflammation in the course of UC. Also, ESR and CRP may be an indicator of an exacerbation of inflammation in the course of IBD. Also, these results ran with *Matsuoka et al.(2018)* who found that white blood cell count , platelet count, CRP and ESR are the most commonly used inflammatory indices in clinical practice for determining IBD activity. Also, *Du et al. (2018)* found that fecal biomarkers (including fecal calprotectin) reflect not only colonic disease but also the upper GIT and small bowel disease activity.

The results of current study showed statistical significant difference between studied groups as regard ulcer, ileitis and edematous mucosa. These results ran with *Weng et al. (2018)* who found that 71% of UC patients with a VTE had pancolitis, and that all CD patients with a VTE had ileocolonic involvement.

The results of current study showed a statistical significant difference between studied groups as regard PLAs.

Results showed statistical significant difference of PLAs as regard disease activity in all studied patients. PLAs were high in severe activity patients followed by moderate activity patients followed by mild activity patients and clinical remission patients. These results ran with *Yoshida et al. (2010)* who found that the more response of neutrophils in forming aggregates with platelets is consistent with neutrophil activation as an important component of the pathophysiology of IBD. *Navaneethan et al. (2010)* found that clinical evidence indicates that abnormalities in both coagulation and platelet function may account for the higher incidence of thromboembolic events detected in patients with IBD. Also, *Schrottmaier et al. (2015)* found that there were associations between PLAs and pro inflammatory effects in numerous pathological conditions, including cardiovascular disease, rheumatoid arthritis and inflammatory bowel disease.

CONCLUSION

In patients with active inflammatory bowel disease (IBD), there were significant high levels of platelet leukocyte aggregates (PLAs) which might be an explanation phenomenon of increased risk for thrombosis.

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تكتل كرات الدم البيضاء مع الصفائح الدموية كعامل خطورة للتجلط في مرضى القولون الإلتهابي

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خلفية البحث: المرضي الذين يعانون من مرض الأمعاء الملتهبة في خطر متزايد للتجلط الوريدي. وتحمل الأحداث الوريدية إعتلالاً ووفيات كبيرة، وارتبطت بنتائج أسوأ في المرضي الذين يعانون من مرض الأمعاء الملتهبة. وقد أشارت الدراسات الي أن طبيعة المرض القابله للتحلل الزائد تنبع من تفاعل معقد بين الانظمة التي تشمل تسلسل التخثر، والاندوثيليوم، والجهاز المناعي، والصفائح الدموية. وبالاضافة الي ذلك، فإن العوامل السريرية التي تزيد من إحتمالية حدوث التجلط الوريدي بين مرضى أمراض الأمعاء الالتهابية تشمل الحمل، والمرض النشط، والمرض الأكثر إتساعاً، والحجز بالمستشفي، والعمليات الجراحية المتصلة بالأمعاء الالتهابية.

الهدف من البحث: تقييم تكتل كرات الدم البيضاء مع الصفائح الدموية في المرضي الذين يعانون من مرض الأمعاء الملتهبة وعلاقته بنشاط المرض.

المرضى وطرق البحث: هذه دراسة جماعية أجريت على 40 حالة من مرضي الأمعاء الملتهبة بالإضافة إلى 20 حالة سليمة تم إدخالهم إلى مستشفى الحسين الجامعي، وبعضهم تم إختيارهم من العيادة الخارجية خلال الفترة من 15 أبريل 2020 إلى 31 مارس 2021. وسجلت من المرضى نتائج الفحص السريري، والنتائج المختبرية، والتنظير القولوني، والتنظير الهيستوباثولوجي، والقياس بجهاز فلوسيتوميتر.

نتائج البحث: كان هناك فارق كبير إحصائياً عالياً بين المجموعات المدروسة فيما يتعلق بنسبة الهيموجلوبين وعدد كرات الدم البيضاء والصفائح الدموية. وقد وجد

فارق كبير إحصائياً بين المجموعات التي خضعت للدراسة فيما يتعلق بسرعة الترسيب. وهناك أيضاً فارق كبير إحصائياً بين المجموعات المدروسة فيما يتعلق بدلائل الإلتهابات ودلائل الإلتهاب البرازي. وقد وجد فارق كبير إحصائياً بين المجموعات المدروسة فيما يتعلق بمجموعات تكتل كرات الدم البيضاء مع الصفائح الدموية.

الاستنتاج: هناك مستويات عالية كبيرة من كرات الدم البيضاء المتكتله مع الصفائح الدموية في المرضى الذين يعانون من مرض الأمعاء الملتهبة النشطة والتي قد تكون بمثابة تفسير لظواهر زيادة خطر الإصابة بالتجلط.

الكلمات الدالة: مرض الأمعاء الملتهبة، تكتل كرات الدم البيضاء مع الصفائح الدموية، التجلط الوريدي.