Mean platelet volume: Can be an indicator to inflammation in spontaneous bacterial peritonitis.

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Abstract: Background and aim: Spontaneous bacterial peritonitis (SBP) is one of serious complications with a high death rate, in cirrhotic patients with ascites. Mean platelet volume (MPV) is an index of active platelets with more proinflammatoryactions. The present study was aiming to prove that high mean platelet volume is an indicator of inflammatory process in SBP. **Subjects and methods:** 50 patients with liver cirrhosis complicated with ascites and 30 healthy subjects as controls.Routine laboratory investigations including complete blood picture (CBC) with MPV estimation, erythrocyte sedimentation rate (ESR), C- reactive protein (CRP), Ascitic fluid analysis, and abdominal ultrasound done for all patients and compared to controls.**Results**: MPV was significantly higher in patients than in controls (11.0±1.2 and 9.9±0.9) respectively with (P.value< 0.001). At a cutoff value ≥ 10.8fl, MPV had 82.5% sensitivity and 100% specificity for determining SBP.**Conclusion**: MPV increases in cirrhotic patients with ascitic fluid infection, indicting occurrence of systemic inflammatory response.
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Keywords: mean platelet volume; ascitic fluid infection; inflammation; cirrhosis.

1. Introduction:

Bacterial infections are common in patients with liver cirrhosis especially in the decompensated phase (1). Spontaneous bacterial peritonitis (SBP) is one of serious complications with a high death rate, occurring in 10% to 30% of cirrhotic patients with ascites, and associated with a very high rate of recurrence (2).

High mean platelet volume (MPV) is an index of larger and more reactive platelets (3), MPV is closely correlated with inflammation and reflects inflammatory burden in different conditions (4).

An increase in MPV has been observed in chronic viral hepatitis because of an increase in the entry of newly produced platelets into circulation, which arelarger in volume than the old platelets (5). MPV is significantly increased in cirrhotic patients with SBP (6).

The study aimed to evaluate whether platelet size changes would be useful in predicting ascitic fluid infection in patients with cirrhosis and comparing it with other inflammatory markers as ESR and CRP.

2. Methods:

2.1. Study population:

A cross sectional (case- control) study was carried out on 50 cirrhotic patients with ascites, they were 24 males and 26 females, their age ranged from (40-72years) with a mean of 55.6 ± 6.7 years. Following results of ascetic fluid culture and PMNL count, Patients were subdivided into two subgroups: sub-group (A) including patients with SBP (40 patients) and sub-group (B) including patients without SBP (10 patients), selected from Kasr El Aini hospital internal medicine department and clinics, Cairo, Egypt. All patients enrolled were confirmed to have liver cirrhosis by clinical and investigatory assessment while patients younger than 18years, with hypertension, diabetes mellitus, heart failure, dyslipidemia or concordant hematological and neoplastic disorder, also patients who were receiving anticoagulant medication, oral contraceptive, non-steroidal anti-inflammatory drugs or antibiotic concomitantly with the study were excluded. Those patients were compared with 30 healthy subjects matching with age.

The study protocol conformed to the ethical guidelines of 1975 the Helinsici declaration and was approved by the ethical committee of internal medicine, faculty of medicine, Cairo University

Written informed consents were obtained from all participants in this study.

2.2. Blood collection and sample preparation:

Blood (10 ml) was withdrawn by venipuncture; After analyzing cell blood counts (CBC) on aliquots of whole blood in EDTA tube (2 ml) using the (sysmexcorp, Kope, Japan) automated hematology analyzer. MPV, platelet distribution width(PDW)& platelet Count were routinely done automatically with CBC analyses by the apparatus, on remainder of the samples (2ml) were collected into a tube containing 0.5 ml of sodium citrate, then the blood was drawn into a Westergren-Katz tube to the 200 mm mark. The tube was placed in a rack in a vertical position for 1 hour at room temperature, at this time the distance

from the lowest point of the curved surface to the upper limit of the red cell sediment (ESR) was measured.

CRP estimation: test was done using AVITEX CRP rapid latex agglutination test kits, by OMEGA DIAGNOSTICS LTD. Omega house, Hill foots Bussiness Village AlvaFK12 5DQ,Scotland, United Kingdom. Liver function tests (bilirubin, Alanine aminotransferase(ALT), Aspartate aminotranseferase(AST), Alkaline phosphatise (ALP), albumin, prothrombin time(PT), prothrombin concentration (PC), & international normalized ratio(INR), urea ,creatinine and lipid profile were measured.

Ascitic fluid samples were aspirated under complete aseptic condition. Ascitic fluid was tapped from each patient and transferred into 2 sterile tubes at least 5ml each. One tube was sent for ascetic fluid analysis and chemistry and the other tube was sent for microbiology laboratory culture. Total leucocytic count was done in well mixed uncentrifuged sample using improved neubauer chamber. The cells were counted in 4 large squares & calculated according to this equation:

$$\text{Number of WBC/ml} = \frac{\text{N. in 4 squares X 10 (depth of chamber)}}{\text{4 (N of chamber)}}$$

Both Leishman and direct Gram films were done from sediment of centrifuged sample after removal of supernatant to make differential count and to identify microorganisms. After centrifugation, the supernatant fluid was subjected to chemical analysis for proteins, glucose and lactate dehydrogenase (LDH).

Three ml of ascitic fluid sample was inoculated directly into 25 ml of tryptic soy broth. Tryptic soy broth was incubated at 37°C and 3 subcultures were done on Blood Agar (BA) and MacConkey Agar (MA) after 24 hours (2nd day), 72 hrs. (4th day) and on the 7th day and the plates were incubated at 37°C. Two ml of the fluid was centrifuged at 1500 rpm for 10 minutes. Supernatant was discarded and deposit was cultured by conventional culture method on Blood agar & Chocolate agar (incubated at 37°C in 5% CO2 atmosphere for 48 hrs.) and MacConkey agar (incubated at 37 °C for 48 hrs.). Another Blood agar plate was inoculated and incubated anaerobically in anaerobic jar for 48 hours.

Patients were categorized into Child grades according to modified Child Pugh classification to assess hepatic functional reserve (Score 5-6 = class A,7-9 = class B, 10-15= class C) (7).

2.3. Statistical analysis:

Pre-coded data was entered into the Statistical Package of Social Science Software program, version 21 (SPSS) to be statistically analyzed. Data were summarized using mean and standard deviation for quantitative variables and frequency and percentage for qualitative ones.

Comparison between groups was performed using Independent sample t-test and Mann Whitney test for quantitative variables and Chi square with Fisher's exact test for qualitative ones. Spearman correlation coefficients were calculated to signify the association between different quantitative variables. P values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

Data were plotted the receiver operating characteristic curve (ROC). Receiver operating characteristic curves were made by arranging the relationship of the true positivity (sensitivity) and the false positivity (1- specificity) at various cut-off points of the tests. If area under curve (AUC) = 1, the index is an ideal predictor, and if AUC = 0.5, the index has no predictive value. The greater the AUC the better the test. The diagnostic accuracy, positive predictive values (PPV), negative predictive values (NPV) sensitivity& specificity were figured & calculated.

3. Results:

Fifty patients with liver cirrhosis due to hepatitis C virus (HCV) infection complicating with ascites were enrolled in this study compared with 30 control subjects, The patients were consisted of24 females (48%) & 26 males(52%). According to ascitic fluid analysis and clinical data, patients were subdivided into subgroup A(SBP) including 40 patients (20 males and 20 females) and subgroup B(non-SBP) of 10 patients (4 males and 6 females).

According to the Child–Turcotte–Pugh score, 13 (26%) patients of Group I were assorted as stage B while 37 (74%) patients were classified as stage C.

Fever and abdominal pain were the main presenting symptoms of patients with SBP subgroup A (100%) followed by encephalopathic changes (22.5%) with no upper gastrointestinal tract bleeding at the time of the study.

The results demonstrates that MPV was statistically significant higher in group I (patients) compared to group II (controls) (11.0±1.2 and 9.9±0.9) respectively (P. value< 0.001), and also subgroup (**A**) had a highly significant difference compared to subgroup (**B)**and healthy controls as regard MPV (11.3 ± 0.9,9.7 ± 1.2& 9.9±0.9) respectively, P. value< 0.001as demonstrated in table (1) and figure (1)

In the current study ESR & CRP were higher in subgroup (**A)**versus subgroup (**B**) and healthy controls with statistical significance (p. value< 0.001) as shown in table(1) and figure(1).

Ascetic fluid polymorph nuclear cells(PMN) and MPV have the highest specificity (100%) with different sensitivity (100% -82.5% respectively) while CRP has higher sensitivity(87.5%) compared to MPV (82.5%) and ESR (77.5%) these were summarized in table (2) and figure (2).

Table (l) Clinical and laboratory data of the study groups

variable	Subgroup A(n=40)(mean ± SD)	Subgroup B (n=10)(mean ± SD)	Control(n=30)(mean ± SD)	t value	P value
Age (years)	55.8 ± 7.2	55.0 ± 4.4	52.6± 6.2	2.0	0.1
WBC ($x10^3/mm^3$)	8.0 ± 3.7	7.4 ± 3.4	7.2 ± 1.8	1.3	0.2
Neutrophil%	63.6 ± 5.9	60.1 ± 6.1	59.1 ± 12.1	1.9	0.1
Lymphocytes %	29.6 ± 5.3	33.4 ± 3.9	32.5 ± 11.2	-1.4	0.2
MPV(7-12fl)	11.0 ± 1.2	9.7 ± 1.2	9.9 ± 0.9	4.5	**<0.001**
PDW(9-18fl)	14.1 ± 2.8	12.9 ± 1.9	13.0 ± 2.7	1.7	0.1 (NS)
Platelet ($x10^3/mm^3$)	106.7 ± 56.6	103.0 ± 50.3	242.9 ± 89.1	8.4	**<0.001**
RBC ($x10^6/mm^3$)	3.4 ± 0.4	3.6 ± 0.4	4.5 ± 0.6	9.7	**<0.001**
HB (g/L)	10.2 ± 1.3	10.7 ± 1.3	12.5 ± 1.4	-7.4	**<0.001**
ESR(mm/hr)	36.4 ± 24.0	19.1 ± 9.3	5.2 ± 1.6	7.1	**<0.001**
CRP(md/dl)	37.8 ± 37.5	12.6 ± 13.7	0.5 ± 0.1	5.4	**<0.001**
A. fluidPMNL(cell/cmm)	672.5 ± 1480.8	139.7 ± 59.1			
INR%	1.7 ± 0.4	1.7 ± 0.3	1.0 ± 0.0	10.4	**<0.001**
Albumin (g/dl)	2.1 ± 0.5	2.3 ± 0.5	4.3 ± 0.7	-15.6	**<0.001**
Bilirubin (mg/dl)	2.8 ± 2.0	3.0 ± 1.7	0.5 ± 0.3	5.1	**<0.001**
AST (Iu/ml)	53.9 ± 27.5	50.3 ± 27.5	29.1 ± 5.5	4.1	**<0.001**
ALT (Iu/ml)	39.3 ± 17.4	36.0 ± 22.2	28.5 ± 7.4	2.7	**<0.001**
ALP (Iu/ml)	78.6 ± 25.2	76.4 ± 17.9	68.4 ± 15.5	1.7	**0.04**
Creatinine (mg/dl)	1.0 ± 0.6	0.9 ± 0.3	0.7± 0.3	2.5	**<0.01**
Urea (mg /dl)	27.3 ± 8.2	28.5 ± 8.4	27.3 ± 4.5	0.0	1.0
Cholesterol(mg /dl)	96.1 ± 28.9	103.9 ± 28.2	152.4 ± 23.2	9.1	**<0.001**

Table (ll): Roc curve results to detect cut off, sensitivity, specificity, PPV, NPV and accuracy of different inflammatory markers (MPV, ESR, CRP &Ascitic fluid PMN) in subgroup A (SBP) & B (Non SBP)

Variable	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	P value	Accuracy
MPV(fl)	≥ 10.8	82.5%	100%	100%	58.8%	0.923	0.848 – 0.997	<0.001	86%
ESR (mm/hr.)	≥ 18.5	77.5%	70%	91.2%	43.8%	0.809	0.673 – 0.944	<0.001	76%
CRP(mg/dl)	≥ 9.0	87.5%	70%	92.1%	58.3%	0.823	0.677 – 0.968	<0.001	84%
fluid PMNL(cell/ccm)	≥ 245	100%	100%	100%	100%	1.0	1.0 – 1.0		100%

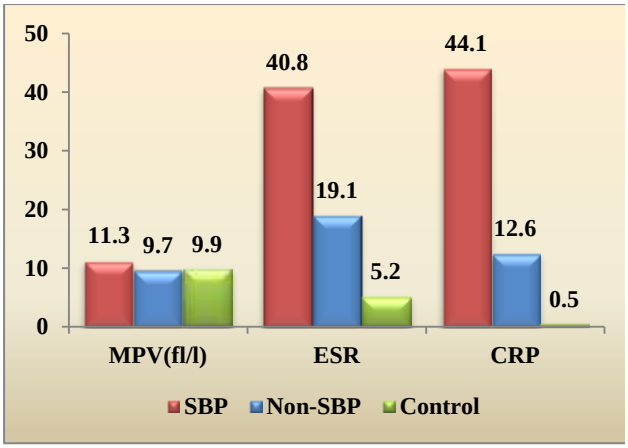

Figure (l): laboratory data of the study groups

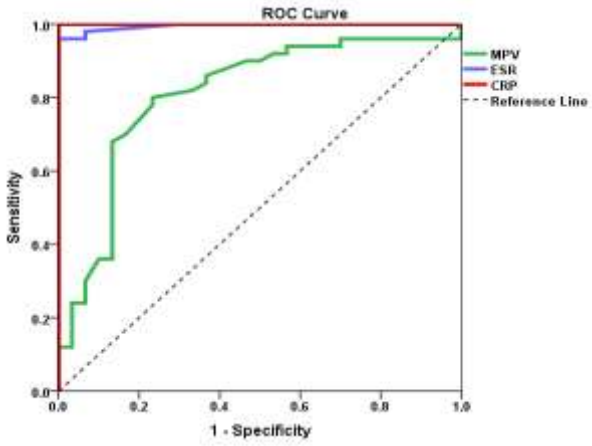

Figure (ll): ROC curve analysis of both group I & II regarding MPV, ESR, CRP &Ascitic fluid PMN

4. Discussion:

Ascitic fluid infection (AFI) is a common and severe complication in cirrhotic ascitic patients (7). Spontaneous bacterial peritonitis (SBP) has a raised rate of morbidity and mortality, occurring in 10% to 30% of cirrhotic patients with ascites, and associated with a very high rate of recurrence (8).

Early detection of SBP is important as the mortality rate among untreated patients is around 50% (9). The diagnosis of SBP was established by ascitic fluid analysis. The most common marker of infection is an ascitic fluid PMN cell count of 250/mm3 or higher (10) and in a meta-analysis, the negative likelihood ratio for SBP if the PMN cell count was greater than 250/mm3 was 0.2 (11).

Despite early initiation of antibiotic therapy, this may result in a satisfactory response in most cases, the mortality still high at 30-50%. (12), so early detection of inflammation has a crucial role for the diagnosis and therapeutic modifications of AFI. Role of platelet as an inflammatory marker in many conditions had been studied, including ascitic fluid infection (AFI). MPV which is considered as an indicator of platelet activation (13), has been reported to be an inflammatory marker and indicator of disease activity in RA & inflammatory bowel disease.

The MPV terms the median platelet size reported in femtoliters and is available on most hematology analyzers. While the PDW gives the volume of the heterogeneity in platelet size either defined as the distribution width at a 20% frequency level or calculated as the SD of platelet volume divided by MPV× 100 (14).

The main goal of the present study was to evaluate mean platelet volume (MPV) changes in cirrhotic patients complicated with ascitic fluid infection.

In the current study MPV was remarkably increased in subgroup A compared with subgroup B (11.3 ± 0.9 and 9.7 ± 1.2) respectively with (P. value < 0.001). MPV was higher in group I compared to group II (11.0±1.2 and 9.9±0.9) respectively and (P. value < 0.001). ESR & CRP were higher in group I than in group II [(36.4±24.0 &37.8 ± 37.5 versus 5.2±1.6&0.5 ± 0.1) respectively with (P value < 0.001). Also ESR & CRP were significantly higher in subgroup A than subgroup B (40.8 ± 24.6& 44.1 ± 39.0 Versus 19.1 ± 9.3 & 12.6 ± 13.7) respectively with (p. value <0.001).

This is in accordance with Suvak et al., who reported a significant increase in MPV in SBP patients than in patients without SBP [8.79±1.01 vs. 8.05±0.83] and in all patients compared to controls [8.37±0.98 vs. 7.88±0.47] respectively with (p. value <0.001).MPV calculation can be considered an accurate diagnostic test in foretellingascitic fluid infection (AFI), possibly because of a progressing systemic inflammatory reaction (12).

This was matching with Abdel-Razik et al, in a study involved 150 patients with cirrhotic ascites (84 patients had AFI and 66 patients did not have AFI) and 70 control participants, there was a significant increase in MPV levels in cirrhotic patients with SBP compared with patients without infected ascites [8.98 ± 0.9 and 8.04 ± 0.86] and in all patients compared to controls [8.37 ± 0.98and 7.88 ± 0.47] respectively with (P<0.001) (13).

PDW was studied to evaluate its role as inflammatory marker the study revealed no significant difference regarding PDW in the compared groups matching with Suvak et al; however it is disagreed with Abdel-Razik et al (12&13).

In subgroup A there were a significant positive correlations between MPV and ESR&CRP and PDW with r. value [0.38, 0.37, 0.64] and P. value (0.02, 0.02, <0.001) respectively, which were not significant in subgroup B.

The current study, ROC analyses found that MPV At a cutoff value of ≥ 10.8fl, had 82.5% sensitivity and 100% specificity for detecting SBP, so MPV is suggested to be used as an easy, cheap, rapid & applicable marker for inflammation specially in ascetic fluid infection independent to the gravity of liver disease as we concluded no significant correlation between MPV and clinical grading of liver disease as indicated by Child pugh score (CPS).

5. Conclusion:

The current study concluded that MPV is a significant test to diagnose systemic inflammation in SBP. At a cutoff value of ≥ 10.8fl, MPV had 82.5% sensitivity and 100% specificity for detecting SBP.

Some limiting points should be mentioned; first the small sample size; so more studies with larger scale are required to confirm the results.Secondly; combined markers are better than single marker to properly evaluate ascetic fluid infection and for more validation. And finally; lacking for the follow up of MPV in patients after treatment to confirm the MPV as an easy, cheap, noninvasive and rapid marker forinflammation.

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